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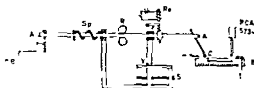


Fig 1 Diagramatic representation of the experimental arrangement used for the mechanical production of rapid stretches B, bath containing the multifibre preparation The bath was filled from below and drained by suction at the top C, clamp holding one tendon of the preparation The other tendon was placed on a hook attached to the anode of the RCA 5734 A--- A movable arm A keying arrangement to prevent angular rotation of the arm is not shown S lead screw to move post V vernier scale to set position of post and thus the amount of stretch Re relay used to release catch at the desired instant R roller bearings to provide for smooth movement of the arm Another set of roller bearings on the other side of the spring Sp is not shown O B, oil bath containing adjustable vane for damping.

The concept of a single fundamental process of activation is based mainly on the results obtained from experiments where muscles were rapidly stretched or allowed to shorten during either isometric twitches or tetanic responses (Hill 1949, 1953, Ritchie 1954) However the same results indicated also that the contractile mechanism is maximally activated at the start of either a twitch or a tetanic response In such circumstances it is possible that both the ability to develop tension and the change in elasticity occur simultaneously It seemed possible, on the other hand, that the two events might be distinguishable under conditions in which the contractile mechanism is only partially activated For this reason the effects of rapid stretches and releases applied during potassium-induced contractures of skeletal muscles were investigated A preliminary report on this investigation was presented at a recent muscle symposium (Frank 1961)

Methods

Preparations These were small bundles of fibres dissected from either the semitendinous or the sartorius muscle of the frog *Rana temporaria* These bundles usually contained 5 to 8 fibres although some had as few as 3 or as many as 10 fibres The cross-sectional dimensions of most of the bundles were determined by measurements made with an optical disk micrometer in the dissecting microscope Bundles of 5 to 8 fibres from the semitendinosus had cross-sectional areas between 1 1 and 4 9 $\times$ 10⁻⁴ cm² (14 preparations) The lengths of several preparations also were measured under various conditions

Preparations from the semitendinosus contained twitch fibres predominantly or exclusively This was routinely checked prior to use by testing the ability of the preparation to respond with vigorous twitches when single electrical stimuli were applied In addition it was noted during testing that the contractures produced by 123 mM KCl lasted only from 10 to 20 sec In several preparations contractures were observed under a dissecting microscope when 123 mM KCl was applied to the slackly held muscle Occasionally a single fibre which failed to relax during a 60 sec exposure was found This was taken as evidence that such fibres were not twitch fibres and they were removed

Many of the experiments involving stretches were repeated with fibre bundles from the sartorius Qualitatively identical results were obtained with preparations from both muscles Preparations from the sartorius were not used routinely because they were more difficult to dissect and less convenient to use

Testing arrangement The bundles were mounted horizontally in the apparatus illustrated diagramatically in Fig 1 The peripheral tendon was clamped to the machine used to stretch the muscle rapidly and the proximal tendon was placed on a hook attached to the anode of the RCA 5734 mechanoelectric transducer valve The transducer valve was fixed to a rod held in a Prior micromanipulator which was used to set the length of the muscle prior to testing The amount of rapid stretch or release could be preset to an accuracy of 0 1 mm with the screw (S)

parallel with the potentiometric recorder and photographed in order to make accurate observations on the early phase of the response Only on oscilloscope recordings were the damped oscillations observed

appropria[illegible] amounts of the choline Ringer's solution with a stock solution containing 494 mM KCl

Procedure The muscles were kept in the oxygenated choline Ringer's solution for at least 12 min between each test with elevated potassium At the start of each experiment the muscle

20 24 C

Experimental range of muscle lengths No attempt was made to find an anatomical or physiological resting length but with only a very few exceptions contractures were recorded and stretches

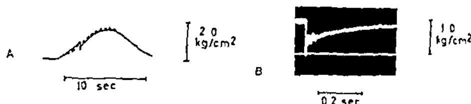


Fig 2 Effect of a rapid release of tension during the rising phase of a submaximum K induced contracture Contractures induced by 35 mM K A-dashed line at + 2 5 mm, A solid line starting at + 3 mm then rapidly released to + 2 5 mm B oscilloscope record made simultaneoulsy with the rapid release in A Semitendinosus Hand-drawn tracings from the original *records are presented in this and subsequent figures* with the exceptions of Fig 2B and 8D which are photographic reproductions of oscilloscope records In all figures only responses obtained in a single experiment on a single preparation are included

with this it was observed that the maximum contracture tension increased as the muscle length was increased over the range of O to + 3 mm and the tensions were about the same or

Results

In their study on single muscle fibres isolated from the frog's semitendinosus Hodgkin and Horowicz (1960) noted that the maximum tensions of contractures induced by 100 or 190 mM K⁺ were consistently larger (about 10 %) than the maximum tetanic tensions in the same fibers Frank (1960) reported a similar finding for small multifibre preparations from the extensor longus digitis IV and the sartorius of the frog In the present study, therefore the maximum contracture tension induced by 123 mM K, *rather than the tetanic response tension, was used as a measure of the maximum tension* that each preparation could develop In most experiments in which all the necessary measurements were made the maximum contracture tensions were within the 3 0 to *4 5 kg/cm² range reported by Hodgkin and Horowicz (1960) for single muscle fibres* (*e g* Figs 3 5 and 7) In those few instances in which slightly smaller maximum tensions were recorded (*e g* Fig 8) it was probable that one or two fibres had been damaged while mounting *the preparations in the bath*

Submaximal contractures were induced by K in concentrations between 27 and 42 mM The size of the contractures produced by any one K concentration varied from preparation to preparation but was reasonably consistent within any one preparation In these submaximal contractures the rising phase of the tension lasted longer than in responses induced by 123 mM K (*e g* Figs 3 5 and 6) The impression gained is that the rate of tension development is so slow that there would be sufficient *time to stretch* adequately any series elastic components in the muscles and that therefore the tensions

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during the rise in tension of submaximal contractures These reductions in lenght were

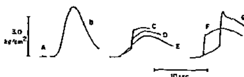


Fig 3 Effects of rapid stretches by different amounts to the same final length (C and D) and by the same amount at different times (F and G) during submaximum K induced contractures

to G contractures induced by 42 mM K C, + 1 to + 3 mm during contracture, D + 2 to + 3 mm during contracture, E contracture at length + 3 mm F and G, + 1 to + 5 mm during contracture

between 0 5 and 2 0 mm During the shortening the tension fell to 0 within a few msec This was followed by a phase of rapid tension development lasting from 200 to 600 msec, after which the muscle continued to develop tension at the same rate as in a contracture produced at the shorter lenght in the same preparation (Fig 2) The maximum tensions recorded were less in contractures with a rapid release to the shorter length than in undisturbed contractures produced at the same shorter length This difference in the maximum tensions decreased with decreasing amounts of shortening and at 0 5 mm it was very small

Resistance to stretch There was only a slight change in the tension recorded when the resting muscle fibre preparations were stretched within the length range described, either slowly manually or rapidly by machine (*e g*, Figs 3A, 5C and 7A) In contrast the same stretch applied during a contracture resulted in a much larger, rapid increase in tension This rapid, large increase in tension for a given stretch, which will be called the resistance to stretch", was followed by a slower change at a rate similar to that occurring prior to the stretch and usually in the same direction

Several factors influenced the size of the "resistance to stretch" For example the resistance to stretch ' increased with increases in the amount of stretch (Fig 3) Figs 3C D and E were submaximal contractures in the same preparation with the preparation at the same length by the end of the contracture In E the muscle was stretched prior to the start of the contracture, in D the muscle was stretched 1 mm during the contracture, to reach the same length and in C the muscle was stretched 2 mm to reach the same final length In Figs 3F and G, the preparation was stretched 4 mm to a final length 2 mm greater than in 3C, D and E It is obvious from Figs 3F and G that the magnitude of the resistance to stretch" varies during the course of a contracture Therefore in

achieved in Figs 3C and D However, it was not possible with the techniques employed in this study to obtain reliably a similar correspondence in all contractures

The resistance to stretch" also seemed to vary with the intensity of activation This was most noticeable with preparations relatively resistant to K⁺ In such cases 27 mM K⁺ produced small but very prolonged contractures and only small responses to rapid stretches were observed When this occurred higher K concentrations were used during the rest of the experiment in order to obtain larger contractures and larger ' resistances to stretch ' Responses no greater than in the unstimulated muscle were obtained when



Fig 4 Production of tension equal to the maximum tension of a 123 mM K⁺-induced contracture by the application ... unstimulated preparation Semitendinosus

the preparations were exposed to 17,5 mM K⁺, a just subthreshold concentration Some of the effects of increasing the K⁺ concentration, and thereby the degree of activation, on the "resistance to stretch" will be described below

As previously mentioned the speed of stretching did not appear to affect greatly the "resistance to stretch", at least within the range of 15 msec taken by the machine to 500 msec or so required for manual stretching

By applying rapid stretches during submaximal contractures it was possible to make the muscles develop or support tensions as large as the maximal tensions obtained during 123 mM K -induced contractures This required a careful adjustment of the various factors affecting the "resistance to stretch", and was done most easily when the submaximal contractures were almost as large as the maximal contractures However, it could be produced also with submaximal contractures only half as large as the maximal (Fig 4)

Variation in the "resistance to stretch" during the course a contracture The remaining factor which was observed to have a pronounced effect on the "resistance to stretch" was the time during the course of a contracture at which the stretch was applied In submaximal contractures the "resistance to stretch", and the tension development without stretching followed different time courses (Fig 5) The "resistance to stretch" was small at the start of the contracture it was maximal about half way through the rising tension phase, and it declined slowly thereafter One result of this time course was that the "resistance to stretch" applied at a small contracture tension was considerably greater at the end than at the start of a contracture After the stretch the tension did not return to the level recorded prior to the stretch but it remained at the new higher level and the contracture continued to progress When stretched during the rise in tension the muscles always developed greater tensions than when the same stretch was applied before exposure to the elevated potassium Fig 5B — dotted record)

Essentially similar results were obtained in experiments with sartorius muscle fibres (Fig 6 There were two minor differences which merit mention Firstly the sartorius appeared to be more sensitive to stretch in that both muscle types developed similar "resistances to stretch" when stretched by the same amount i e, 3 mm) even though the "0" length of the sartorius was about twice that of the semitendinosus Secondly the "resistance to stretch" appeared to decline more slowly as the contracture proceeded in the sartorius and in this muscle the "resistance to stretch" at the end of the contracture was relatively greater than in the semitendinosus

In contrast with the results obtained in submaximal contractures the "resistance to stretch" in contractures induced by 123 mM K was maximal near the start it declined slowly during the rise in tension and rapidly thereafter Fig 7C This time course of



Fig 5 The "resistance to stretch" at various times during submaximum K induced contractures of a semitendinosus muscle preparation A contracture induced by 123 mM K at + 3 mm B, contracture induced by 27 mM K+ dotted line at + 3 mm, solid lines starting at "O" and then rapidly *stretched to + 3 mm The final portions of* each of the latter have been left out. C, rapid stretch from "O" to + 3 mm in the unstimulated preparation

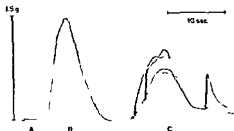


Fig 6 The "resistance to stretch" at various times during submaximum K+ induced contractures of a sartorius muscle preparation A, rapid stretch from "O" to + 3 mm in the unstimulated preparation B contracture induced by 123 mM K at + 3 mm C contractures induced by 42 mM K dashed line at + 3 mm solid lines starting at "O" and then rapidly stretched to + 3 mm In the latter the "resistances to stretch" have been artificially intensified in order to make them readily distinguishable from the slower changes in tension

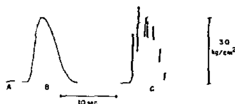


Fig 7 The "resistance to stretch" at various times during maximum K induced contractures of a semitendinosus muscle preparation A rapid stretch from "O" to + 3 mm in the unstimulated preparation B and C contractures induced by 123 mM K B at + 3 mm, C several starting at "O" and then rapidly stretched to + 3 mm In C only the very start of the contractures shown the "resistances to stretch" are shown by intensified upstrokes, and in each case a bit of the subsequent record is shown by the dashed lines

events might have been expected from the results of other investigators who have studied the time course of activation during twitches and tetani (Hill, 1949, Ritchie, 1954) However there was one unexpected observation In most, but not all, experiments of this type there was a period of active tension development following the initial increase in tension which resulted from the rapid stretches applied near the end of the contractures (Fig 8) The course of this tension development is seen without the inherent response lag of the potentiometric recorder only on oscilloscope records (Fig 8D) The tension does not fall *immediately after the rapid events associated with the stretch* (about 20 msec) It rises instead, reaching a maximum at about 300 msec, and only after about 600 msec does it fall at a rate similar to that prior to the stretch This period of active tension development was observed also in some experiments where stretches applied near the end of the maximal contractures resulted in a "resistance to stretch"

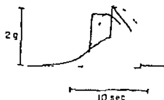


Fig 4 Production of tension equal to the maximum tension of a 123 mM K^+-induced contracture by the application of a rapid stretch during a submaximum K^+-induced [illegible]

unstimulated preparation Semitendinosus

the preparations were exposed to 17,5 mM K^+, a just subthreshold concentration Some of the effects of increasing the K^+ concentration, and thereby the degree of activation, on the "resistance to stretch" will be described below

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Variation in the "resistance to stretch" during the course a contracture The remaining factor which was observed to have a pronounced effect on the "resistance to stretch" was the time during the course of a contracture at which the stretch was applied In submaximal contractures, the "resistance to stretch", and the tension development without stretching followed different time courses (Fig 5) The "resistance to stretch" was small at the start of the contracture, it was maximal about half way through the rising tension phase, and it declined slowly thereafter One result of this time course was that the "resistance to stretch" applied at a small contracture tension was considerably greater at the end than at the start of a contracture After the stretch the tension did not return to the level recorded prior to the stretch but it remained at the new higher level and the contracture continued to progress When stretched during the rise in tension the muscles always developed greater tensions than when the same stretch was applied before exposure to the elevated potassium (Fig 5B — dotted record)

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In contrast with the results obtained in submaximal contractures, the "resistance to stretch" in contractures induced by 123 mM K^+ was maximal near the start, it declined slowly during the rise in tension and rapidly thereafter (Fig 7C) This time course of



Fig 5 The "resistance to stretch" at various times during submaximum K induced contractures of a semitendinosus muscle preparation A contracture induced by 123 mM K at +3 mm. B contracture induced by 27 mM K dotted line at +3 mm solid lines starting at "O" and then rapidly stretched to +3 mm The final portions of each of the latter have been left out. C, rapid stretch from "O" to +3 mm in the unstimulated preparation

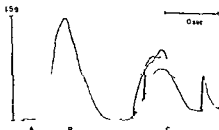


Fig 6 The "resistance to stretch" at various times during submaximum K induced contractures of a sartorius muscle preparation. A rapid stretch from O to +3 mm in the unstimulated preparation B contracture induced by 123 mM K at +3 mm C, contractures induced by 4? mM K dashed line at +3 mm solid lines starting at "O" and then rapidly stretched to +3 mm In the latter the "resistances to stretch" have been artificially intensified in order to make them readily distinguishable from the slower changes in tension.

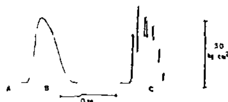


Fig 7 The resistance to stretch at various times during maximum K induced contractures of a semitendinosus muscle preparation A rapid stretch from "O" to +3 mm in the unstimulated preparation B and C contractures induced by 123 mM K B at +3 mm C several starting at O and then rapidly stretched to +3 mm In C only the very start of the contractures shown the "resistances to stretch" are shown by intensified upstrokes and in each case a bit of the subsequent record is shown by the dashed lines

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Fig 8 Initiation of active tension development by the application of a rapid stretch near the end of a maximum K⁺ induced contracture A, rapid stretch from 'O" to + 3 mm in the un stimulated preparation B and C, contractures induced by 123 mM K⁺ B, at + 3 mm, C, starting at O" and then rapidly stretched to + 3 mm The 'resistance to stretch" in C has been artificially intensified D, oscilloscope record made simultaneously with the rapid stretch n C Semitendinosus

no greater than in the resting muscle Occasionally this period of active tension development lasted for as long as 1 sec but usually it lasted about as long as or somewhat less than in the test shown in Fig 8

Discussion

It is mainly from the works of Hill (1949, 1953) that present concepts are drawn of the mechanical events which occur during an isometric twitch response of skeletal muscle Two main lines of evidence are used to support the view that during the latent period the internal contractile apparatus rapidly becomes completely activated and fully capable of developing the maximal tetanic tension First, if the series elastic component is made taut by stretching the muscle rapidly shortly after a twitch-inducing stimulus the muscle will develop a tension equal to the maximum tetanic tension It is known, however, that during activation the contractile apparatus itself also undergoes an increase in elasticity, or as it has been called here, an increased 'resistance to stretch' Hence the interpretation of these results is based on the assumption that the change in elasticity of the contractile element develops in parallel with the change in its ability to develop tension following a stimulus Thus activation is conceived of as a single fundamental process composed of these two events Second the rate of tension development is the same at the start of either a twitch or tetanus More recently, however, Jewell and Wilkie (1958) have found that the redevelopment of tension following quick release during a tetanus is more rapid than the initial development of tension at the start of the tetanus This implies that the active state takes a considerable period of time to become fully developed

The basis of the experimental approach in the present study is the possibility that if activation is the result of two distinct processes, it might be possible to distinguish these processes readily during submaximal potassium-induce contractures in which the whole process of tension development is greatly prolonged and in which presumably only a partial activation of the contractile mechanism is achieved This concept was tested by the application of quick releases during the rising phase of tension development in sub maximal potassium-inducted contractures (Fig 2) Even ignoring the very rapid phase

of tension development which lasts for a few msec after the quick release, there is still a period lasting from 200 to 600 msec during which the tension rises at a rate much greater than that found at any time during the unreleased contracture It would seem that the contractile mechanism is capable of stretching the series elastic component at a rate much greater than it does during these contractures and therefore the tension developed truly reflects the maximum ability of the contractile mechanism to develop tension at any instant during the contracture

It should be pointed out however, that this condition can be obtained only when preparations containing very few muscle fibres are used because in large multifibre preparations or in whole muscles the maximum tension is reduced due to the slow diffusion of potassium into the muscle (Frank 1960)

The most significant finding in the present study probably is that the "resistance to stretch" and the development of tension follow two distinctly different time courses during submaximum potassium induced contractures (Figs 5 and 6) Both increase during the start of the contracture but at different rates However, the difference in time course is even more prominent during the decline of the contracture tension at which time the resistance to stretch' remains quite large even when the tension has practically disappeared If as is suggested by the work of Hill (1949), the total tension recorded very shortly after a rapid stretch is a measurement of the active state of the contractile mechanism then it is difficult to see why the rate of tension development recorded at the stretched length (Fig 5B — dotted record) begins to decline at a time corresponding to when this measure of the active state is still increasing (Fig 5B, tensions at the end of the second and third rapid stretches), why the tension recorded declines more rapidly than this presumed measure of the active state and finally why this measure of the active state is so much larger near the end of a contracture than it is at the same contracture tension near the start of the contracture (Figs 5B and 6C) The most obvious explanation of these discrepancies is that the total tension recorded following a rapid stretch is not a measurement of a single process of activation but rather the resultant of two processes, one being the ability to develop tension just prior to the stretch as indicated by the actual tension developed at that instant and the other being the resistance to stretch of the contractile component as indicated by the additional tension developed following the stretch

One of the corollaries derived from the concept that activation is a single fundamental process is that the tension recorded during a response at a time when the tension is neither increasing nor decreasing (i e at a plateau) is a measure of the active state (Hill 1949 Ritchie 1954) Several such tension plateaus were obtained during the experiment illustrated in Fig 5B Tension plateaus were recorded approximately half a second after each of the first two stretches in Fig 5B The tension plateau was much larger after the second rapid stretch Nevertheless, the rate of tension development at the stretched length as indicated by the dotted line record was about the same at these two points during the contracture Following the second rapid stretch two such plateaus are evident Even though the total tension and therefore presumably the active state was larger at the second plateau the rate of tension development has declined greatly at the corresponding instant during the contracture at the stretched length Finally if the maximum tension developed during the contracture at the stretched length (dotted curve is also a measure of the active state it is difficult to see how the tensions at corresponding instants could be larger in the responses following the first two rapid stretches in the same preparation if the only effect of these rapid stretches was to stretch the series elastic

component and thereby give a truer picture of the actual state of activation during the contractures

The results obtained from the application of rapid stretches during contractures induced by isotonic KCl (Fig 7) were much closer to those that might have been expected from currently accepted concepts However, even in maximal contractures the rate of tension development is still very slow and is likely to be a reliable measure of the maximum ability of the muscle to develop tension at each instant Hence it would appear that in maximal contractures the ' resistance to stretch ' develops more rapidly than the ability to develop tension

The picture that emerges from the results of the present study, and from the results of the other studies discussed here, is that after a stimulus at least two distinct processes occur in the contractile mechanism Together these processes constitute what is currently called activation" or the active state" One process is the development of an increase in elasticity or the resistance to stretch", presumably as a result of the formation of linkages between actin and myosin filaments, and the other process is an increase in the ability to develop tension or to shorten as the two sets of filaments move past each other presumably by the breaking and reformation of the links at different points along the filaments The increase in elasticity develops very rapidly at the start of a twitch or tetanus reaching a maximum during the latent period (Hill 1949, 1950), whereas the ability to develop maximum tension proceeds much more slowly (Jewell and Wilkie 1958) During contractures these two processes are spread out greatly in time and can be readily distinguished In addition the relative time courses of these two processes can be varied by changing the concentration of potassium used to induce the contractures

Acknowledgments

I wish to thank Professor Stephen Thesleff for making the facilities of his laboratory available to me and he and Dr Carl Pinsky of Winnipeg Canada for their helpful comments and suggestions This work was sponsored by the Air Force Office of Scientific Research OAR through the European Office Airospace Research United States Air Force

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From the Surgical Research Laboratory II, Department of Surgery, University of Washington School of Medicine, Seattle, Wash., U. S. A.[1]

The Effect of Antral Acidification on the Intestinal Phase of Gastric Secretion in the Heidenhain Pouch Dog

By

LLOYD M. NYHUS, MARCEL J. RHEAULT[1] and LARS S. SEMB[2]

Received 7 December 1964

Abstract

Nyhus L. M., M. J. Rheault and L. S. Semb. *The effect of antral acidification on the intestinal phase of gastric secretion in the Heidenhain pouch dog.* Acta physiol. scand. 1965. 65. 11—19. — Five dogs [illegible]

carried out

I. Test meal, no perfusion of antrum 25 expts. in 5 dogs [illegible]

dogs, and the antrum perfused with saline, there was a release of endogenous gastrin from the an[illegible]

pre[illegible]

inhibitory effect on release of gastrin

There is still controversy in the literature whether the inhibitory effect on gastric secretion by acidification of the gastric antrum is due to an antral chalone (Margolos and Harrison 1956, Harrison, Lakey and Hyde 1956, Jordan and Sand 1957, Shimizu, Morrison and Harrison 1958, Danhof 1960, DuVal and Price 1960, 1961, DuVal, Fagella and Price

[1] Research Associate, R. Samuel McLaughlin Travelling fellow, on leave from Montreal, Quebec, Canada.
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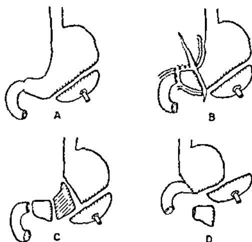


Fig 1 Operative procedures *A* Heidenhain pouch is prepared in usual manner *B* Vagally denervated separated antral pouch is also formed The lesser curvature of the antrum is cleaned completely of all tissue, and the lesser omentum is sectioned transversely *C* To avoid any antral tissue remaining in gastric remnant a partial distal gastrectomy is performed removing nearly all of the lesser curvature *D* Gastrointestinal continuity is restored by gastroduodenostomy

1961, Thompson *et al* 1962 a, b) or an inhibition of gastrin release (Oberhelman *et al* 1952, [illegible]
1960, [illegible]
Gillesp [illegible]

In a recent communication from this laboratory (Rheault *et al* 1965), acidification of the isolated, vagally denervated antrum in dogs with Heidenhain pouches, failed to inhibit histamine — as well as gastrin — induced secretion from the Heidenhain pouches However, gastrin released from the isolated antrum by topical application of acetyl choline, was depressed markedly by lowering the pH of the antrum to about 1 5 It was, therefore, concluded that the most probable effect of antral acidification was one of inhibition of gastrin release, rather than one of releasing an antral chalone

On the other hand, it was felt that an antral chalone, if produced in these experiments, might not be of sufficient strength or was in other ways ineffective to counteract the stimulatory effects of exogenous histamine or gastrin and would therefore not be demonstrable by the technique employed The experiments to be reported here were therefore designed to study the effect of antral acidification on gastric secretion stimulated by endogenous secretogogues released by feeding (intestinal phase)

Materials and methods

Animals

5 mongrel dogs weighing from 8—21 kg were prepared with Heidenhain pouches in the manner reported from this laboratory (De Vito and Harkins 1959) The fundic pouches were cannulated to the exterior with a tight fitting stainless steel cannula (Condon and Harkins 1962) A vagally denervated antral [illegible]
junction between f [illegible]
incisura angularis [illegible]
of the right and lef [illegible]
closed in two layers [illegible] chromic catgut and continous 00 black silk [illegible] end was brought to the exterior as a fistula To secure vagal denervation of the antrum the pouch was made devoid of all tissue leading to the lesser curve and the gastrohepatic ligament was transsected The blood supply to the pouch was secured by the right gastroepiploic artery and vein Gastrointestinal continuity was reestablished by gastroduodenostomy in two layers after

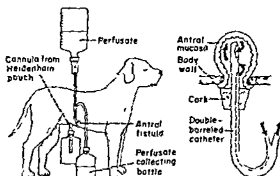


Fig 2 The experimental arrangement in perfusion of antral pouch (left) The perfusates (NaCl and HCl) come from a bottle with a drop-counting device and are led to the antrum through a soft rubber catheter The perfusates are drained from the antrum through another channel in the catheter and collected in another bottle A schematic drawing of the antral pouch and the catheter is shown to the right

a sleeve of gastric mucosa was resected to insure that no antral mucosa was left in the remnant stomach (Fig 1) In one of the dogs (Dog no 21), the remnant stomach was completely removed and an oesophagoduodenostomy was performed The dogs were allowed to recover from the

Experiments

The 5 dogs were subjected to 4 different types of experiments and each type of expreiment was repeated from 2 to 5 times in each animal (Table 1)

Secretory tests

After recovery from the operations the dogs were trained to stand in a Pavlov frame for periods up to 8 hrs

On the day of the test after a 14—18hr fasting period where only water *ad libitum* was allowed, the dogs were brought to the frame and a 1 hr period was allowed to elapse to ascertain that there was no secretion from the Heidenhain pouches At the end of the 1 hr period the test meal was given and the gastric secretion was collected in half hour samples for 4 hrs

Test meal

200 g of commercially produced strained beef liver homogenate ("Gerbers" Armours Ltd) was put on a tray and the dogs ate this with good appetite in a matter of a few minutes If for some reason the meal was not eaten within 5 min after it had been served to the dog the test was discontinued for this animal that day

Antral perfusion

Half an hour before the test meal was given a thin two-way catheter was fitted to the antral pouch in the way earlier described (Shapira Morgenstern and State 1960, Rheault *et al* 1964) (Fig 2) A slow perfusion (125 ml/hr) of either 0 1 N HCl or 0 9 % saline was started one half

the animals were fasting and 3 hrs after a meal

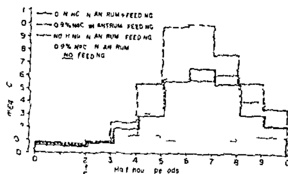


Fig 3 Average half hour output of acid in Heidenhain pouch dogs after a test meal The meal was given at F, after a 1 hr base-line collection period The acid output after I) a meal alone is indicated by II) a meal and the antrum perfused with saline (- - -) and III) a test meal and the antrum perfused with 0 1 N HCl (————) The average acid response per half hour when the antrum was perfused with saline and no meal was given is also shown ( ) The acidity of the 10 cc of 0 005 N HCl that was added to each half hour collection of acid in the Heidenhain pouch has *not* been subtracted from the values shown in the figure Therefore the zero secretion corresponds to the 0 05 meq HCl line

TABLE I Four hour gastric acid response to test meal in Heidenhain pouch dogs

Dog No	Average output of acid in mEq ± SD[1] Exp I Test meal No perfusion of antrum	Exp II Test meal Perfusion of antrum $\bar{c}$ 0 9 % NaCl	Exp III Test meal Perfusion of antrum $\bar{c}$ 0 1 N HCl	Exp IV No test meal Perfusion of antrum $\bar{c}$ 0 9 % NaCl
2	4 30 ± 0 77 (4)[2]	5 03 ± 0 82 (5)	5 46 ± 0 90 (5)	1 34 ± 0 28 (2)
9	2 55 ± 1 28 (5)	2 96 ± 0 93 (5)	1 63 ± 0 69 (5)	0 91 ± 0 00 (2)
15	0 90 ± 0 18 (5)	1 26 ± 0 49 (5)	0 81 ± 0 25 (5)	0 43 ± 0 02 (3)
18	6 39 ± 2 04 (5)	10 77 ± 2 93 (5)	4 88 ± 2 65 (5)	1 67 ± 0 93 (3)
21	1 44 ± 0 86 (5)	2 65 ± 0 79 (5)	1 29 ± 0 66 (5)	No obs
Grand Mean	3 07 ± 2 40 (24)	4 53 ± 3 67 (25)	2 81 ± 2 25 (25)	1 07 ± 0 87 (10)

[1] SD Standard deviation
[2] —2 log (p) 2 loge (1/p rest of the group

Acidity of gastric juice

The secretory response of the Heidenhain pouch to the test meal was determined in half hour samples Gastric juice was collected by a technique described from this laboratory (Savage *et al* 1963) In essence this consists of washing out the fundic pouch with a very weak acid solution (10 ml of 0 005 N HCl) at every collection The contents of hydrochloric acid were determined by titration against 0 01 N NaOH in a Fisher Automatic Titrimeter Model 36 to pH 7 0 (titratable acid) In the tables and figures the secretory responses are expressed as meq of titratable acid The acidity of the added HCl (0 05 meq at every half hour collection) has not been subtracted from the figures reported

The gastric secretory responses to 100 μg of histamine acid phosphate or 5 mg of gastrin[1] (Fletcher *et al* 1961 Anderson *et al* 1961) in most of the dogs were compiled from the data of an earlier communication (Rheault *et al* 1964) but some were recorded separately (Table II)

Statistical analyses

The results were subjected to statistical analyses according to Fisher (1958) utilizing the "Student s t test" to evaluate the significance of means and the chi square distributions utilizing the test for "Significance of the product of a number of independent probabilities The tables of Pearson and Hartley (1956) were used

[1] The gastrin was kindly supplied by T L. Fletcher, Ph D It was made from an alkaline extract of porcine antral mucosa and was considerably purer and more potent than that described in the preliminary communications

Statistical comparison of groups

I vs II			II vs III			III vs I		
t	p	—2 log p[3]	t	p	—2 log p[3]	t	p	—2 log p[3]
1 443	0 117	4 29	0 79	0 25	2 76	2 16	0 04	6 44[4]
0 580	0 32	2 37	2 58	0 02	7 73	1 47	0 09	4 84
1 53	0 12	4 33	1 81	0 05	6 03	0 64	0 06	5 63
2 75	0 02	7 73	3 33	0 001	13 45	1 00	0 25	2 77
2 37	0 07	5 21	2 96	0 01	8 54	0 31	0 41	1 78
χ^2 23 87 10 DF p ~ 0 008			χ^2 38 51 10 DF p 0 00004			χ^2 15 02 8 DF p 0 059		

[3] The numbers in parentheses refer to the number of experiments

[4] This result was not included in the computation for χ^2 since the polarity is opposite to the

TABLE II The gastric acid output in Heidenhain pouch dogs after a test meal, or injections of histamine or gastrin subcutaneously 4

Dog No	Test meal 200 g beef liver[1]	Histamine 100 μg subcut [2]	Gastrin 5 mg subcut.[3]
2	3 57—5 19[4] (5)[5]	not obs	2 17—4 62 (5)
9	0 67—3 73 (5)	3 50 (1)	not obs
15	0 78—1 22 (5)	1 14—2 52 (5)	2 16—3 47 (5)
18	4 23—8 61 (5)	1 40—6 54 (5)	2 91—5 43 (5)
21	0 46—2 49 (5)	3 01—4 66 (6)	0 40—1 00 (8)

[1] Four hour gastric acid output following a test meal of strained beef liver (Gerbers)
[2] Two-hour gastric acid output following a single injection of 100 μg histamine acid phosphate subcutaneously
[3] Two-hour gastric acid output following a single injection of 5 mg of gastrin subcutaneously
[4] All values given represent the range of observed values in the experiments
[5] The numbers in parantheses indicate the number of experiments from which the information is derived

Results

In Fig 3, the average acid output per half hour after feeding of a standard test meal is shown, and in *Table I a summary is given of the total acid output for all groups of* experiments

In all the groups of experiments where the test meal was given, a gastric secretion was evoked It started in the second half hour period after the meal was given, increased gradually and reached maximal values in the fourth through the sixth half hour period and after that fell gradually and approached baseline values after 4 hrs (Fig 3)

There was a significantly larger average output of acid from the Heidenhain pouches in 5 dogs when their antra were perfused with saline (exp II) as compared to the acid output when the antra were acidified (exp III) or not treated (exp I) There was no significant difference between the average acid output after a test meal when the antrum was not treated (exp I) and when the antrum was acidified (exp III) in 4 of the 5 animals, and a barely significant higher output in the fifth animal (Dog no 2) Perfusion of the antrum with 0 9 % saline when no test meal was given (exp IV), evoked a small and relatively constant acid output from the Heidenhain pouches in 4 dogs The average acid output under these conditions was smaller than the output in expts I through III, but greater than the zero values observed in the 1 hr period before perfusion of the antrum was started

TABLE III pH Measurements in antral pouches and antral perfusates

	Antral (direct)[1]		Antral perfusates	
Dog No	Fasting[2]	Food stim [3]	NaCl	HCl
2	7 2—7 8	7 2—7 6	5 7—6 7	1 0—1 5
9	6 3—6 7	4 8—5 3	5 7—6 7	1 0—1 5
15	7 2—7 8	6 3—6 7	5 7—6 7	1 0—1 5
18	8 2—8 4	4 6—4 8	5 7—6 7	1 0—1 5
21	8 4—8 8	6 8—7 2	5 7—6 7	1 0—1 5

[1] pHydrion paper
[2] In the morning before food was given, after an overnight fast.
[3] Three hours after a regular meal was given

The pH of the saline antral perfusates was between 5 7 and 6 5, for the HCl perfusates pH was between 1 0 and 1 5 The intra-antral pH, measured directly by indicator paper, was observed to be between 6 3 and 8 8 in the fasting animals, and between 4 6 and 7 6 3 hrs after a meal (Table III)

Discussion

The test meal employed in these studies seems to be an adequate stimulus for the gastric acid secretion from the Heidenhain pouches, since the range of gastric outputs *after the test meal was comparable to the various ranges of acid output encountered* when histamine or gastrin was injected subcutaneously in the same dogs (Table II) Furthermore, the gastric acid response to our test meal is also comparable to the findings of others utilizing a similar type of experimental preparation, but a different and more bulky meal (Thompson *et al* 1962b)

Since the antrum was excluded from gastro-intestinal continuity, and further was vagally denervated, a major portion of the cephalic (vagal), vagal antral and local antral (Nyhus *et al* 1960) phases *of gastric secretion was excluded Thus the meal* responses observed should probably represent true changes in the intestinal phase of gastric secretion

was acidified to pH < 1 5 (exp III) was significantly less than, when under similar conditions the antrum was perfused with saline (exp II) This decrease would *seem to* indicate that acidification of the denervated, isolated antrum had an effect upon the intestinal phase of gastric secretion suggesting that an antral chalone had been released *by acidification, as reported by* others (Margolus and Harrison 1957, Thompson *et al* 1962) Is there a resolution to these seemingly inconsistent observations found within our experiments? A close look at all the results helps to delineate an answer

When saline is perfused through the antral pouch, the pH of the perfusate is shown to be between 5 7 and 6 7 in our animals At this pH the release of gastrin is enhanced,

even to small stimuli Threshold or subthreshold amounts of exogenous gastrin increase the response to intestinal phase stimulation of gastric secretion This increased reactivity of the parietal cells by exogenous gastrin could not be altered by antral acidification (Andersson and Olbe 1964, Jordan and De LaRosa 1964)

Perfusion of the denervated antrum with 0 9 % saline in the experiments reported herein, releases gastrin from the antrum (exp IV) as indicated by detectable secretion from the Heidenhain pouches Therefore the increased response to saline antral perfusion and feeding relates to the augmentation phenomenon just discussed The augmentation is not seen in expts I ("pure" intestinal phase) since no endogenous gastrin is being released, nor is it seen in four of five animals in expts III, because antral acidification blocks the release of endogenous gastrin

In a fifth animal (Dog no 2), there was a 27 per cent increase in the output of acid after the meal, upon acidification of the antrum ($p = 0.04$) This we have no explanation for, except that possibly the acid perfusion could have been incomplete during some of the experiments, and not all the antral mucosa was bathed in acid, thereby causing some release of gastrin (Andersson and Olbe 1964)

On the basis of these experiments, acidification of the gastric antrum seems to have no effect on the intestinal phase of gastric secretion, and the concept of release of an antral chalone, active againts intestinal phase stimulation of gastric secretion cannot be supported

We wish to thank the chairman of the Department Professor Henry N Harkins M D Ph D, for his kind interest inspiring directions and valuable suggestions regarding this project and also for the excellent facilities he has provided for us Mr A Gajewski and Mr Joel Baker Jr

staff for their help with the titrations

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The Kidney Cortex Slice Technique as a Model for Sodium Transport In Vivo

A Qualitative Evaluation

By

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Abstract

cellular concentrations in the functioning cells, which were found to be 4—15 μeq Na^+/g cellular water and 125—135 μeq K^+/g cellular water Efflux studies on slices incubated with radioactive sodium did not disclose any active component when flushed with Ringer solutions of different electrolyte composition and temperatures, or in the presence of metabolic inhibitors At a sulfate concentration of 6 mM, at which concentration the volume of distribution

over rate of intracellular sodium of striated muscles

Slices of mammalian kidney cortex are capable of maintaining low sodium high potassium concentrations relative to those of the medium when incubated in Ringer solutions (e g Mudge 1951 a, Whittam and Davies 1953, Davies 1954) The magnitude of these concentration differences (about 30—50 meqv/l) are correlated to the metabolism of the slices since changes may be induced by addition of metabolic inhibitors, changes in temperature, aerobic — anaerobic conditions etc, suggesting that the differences are due to active ion transport In accordance with this suggestion Whittembury (1961) has obtained intracellular electrical potential differences of 50—60 mV (cell negative)

om superficial slices of guinea pig cortex in most of impaled cells suggesting proximal ocalization of the electrode (pers comm)

When pieces of tissue in vitro are used for determination of flux rates of ions across ellular membranes the diffusion in the extracellular space represents a complication o the exact estimation, a complication which may be minimized by reducing the size of the piece of tissue used (Keynes 1954) The use of very thin slices was introduced by assen and Thaysen (1961) for studies of the efflux rate of sodium from isolated renal ortical tissue which furthermore was preeluated at low temperature in order to obtain *a correction for passive leakage It was inferred from an apparently rather high Q_{10} that* active ' sodium extrusion was the limiting factor for the eluation rate observed ollowing a rapid shift in temperature from zero to 37° C

From in vivo studies a direct inhibitory effect of angiotensin on the proximal tubular unction of rat kidneys has been demonstrated by observations of the rate of reabsorption of luminal fluid after cessation of filtration (Leyssac 1963 b) This in vivo effect differed markedly from the apparent in vitro effect demonstrated in preliminary studies (Leyssac, Lassen and Thaysen 1961) using the slice technique of Lassen and Thaysen The inhibition in slices in vitro seemed to be a complete blocking of any active ' transport of sodium whereas the effect in vivo was limited, being an inhibition of only 50—65 per cent of the maximal spontaneous transport rate When, however the in vitro effect was re investigated by means of an improved recording technique it was impossible to demonstrate any significant effect of angiotensin nor of other known in hibitors The present investigation, the purpose of which was to clarify the reason (s) for this discrepancy, has indicated that transcellular transport of sodium can not possibly take place in slices and that the bulk of the intratubular sodium is exchanged with sodium in the medium by diffusion only, obscuring any possible active" sodium transport from a small true intracellular sodium pool

Methods

Apparatus

peratures could be shifted from zero to 40 C in less than one minute The coils terminated in a

recorded on a FHK 1/542 recorder

counter with thin window

Efflux studies

a)

with 5 mM potassium phosphate, oxygenated and containing glucose and α ketoglutarate. After 5—10 min the slices were transferred to a similar Ringer solution containing 20—30 μC ^{22}Na in either 1.5 or 2 ml of the solution. Slices were incubated at either room temperature or 37° C for 15 min. After incubation a slice was lightly blotted on sucking paper, suspended on the hook, and the washing chamber, filled with inactive medium, was screwed on and placed in the well-counter. The rate of loss of activity was recorded during flushing with non radioactive media at either zero ° C or at 37° C.

b) In experiments in which simultaneous efflux of ^{22}Na and $^{35}SO_4$ was measured Ringer solutions contained 6—10 mM sulfate. 20—30 μC ^{22}Na and 500 μC ^{35}S-sulfate was added to the incubation medium, and slices were incubated when suspended in the washing chamber.

c)

Registration of efflux rate

In all experiments the activity (^{22}Na) remaining in the slices was directly recorded from the ratemeter during the wash-out period. The logarithm of the recorded count rates, corrected for background, was plotted as the ordinate against time in minutes along the abscissa to give the efflux curves.

In experiments with simultaneous efflux of ^{22}Na and $^{35}SO_4$ the logarithm of counts per min of both isotopes in the successive samples of the wash-out medium collected over periods of 5 sec was plotted (ordinate) against time (abscissa).

Uptake studies

tion, 1 ml of supernatant was analyzed for ^{22}Na and $^{35}SO_4$, as described above, and compared with those of the diluted incubation medium.

Tissue electrolytes

°C and at this temperature ... ighed ... HNO_3 ... super- ... s and ... ndard



Fig 1 Fig 2

Fig 1 Section of rat kidney cortex frozen at the instant of removal (Thickness 3 μ, magnification × 450)

Fig 2 Section of rat kidney cortex slice incubated at 37° C for 15 min in Ringer solution (Thickness 3 μ, magnification × 450)

For analyses of tissue electrolytes special series of standards were prepared both for sodium and for potassium at different fixed concentrations of potassium and sodium respectively Sodium standards with a potassium concentration as close to that in the sample, as indicated from preliminary determinations, were chosen A similar procedure was used for selection of suitable potassium standards, which were chosen with a sodium concentration close to that in the sample

isopentane cooled to about — 160° C Frozen cortical tissue was separated at — 70 C and weighed Blood samples were centrifuged at once and serum was separated Cortical tissues and 100 μl of corresponding sera in 1 ml water were counted in the well scintillation counter and the amount of plasma per gramme cortex was calculated as a measure of the plasma space

Histological technique

Rat kidneys were snap-frozen either immediately after cutting the renal pedicle of the anaesthetized laparotomized animal or 20—60 sec after clamping the pedicle and removal of the kidney A kidney cortical slice was prepared as described above and incubated in oxygenated Krebs-Robinson Ringer buffered with potassium phosphate (5 mM) to pH 7 4—7 5, containing glucose (100 mg per 100 ml) at 37° C for 15 min After incubation the slice was frozen Frozen tissues were transferred to absolute ethanol at — 20 C and left in this temperature for 6—12 days (freeze substitution) After freeze substitution tissues were embedded in paraffin, sectioned and stained as described in a previous paper (Levssac 1963 a)

Cellular area

The histological sections of snap-frozen cortical tissues were examined in a Reichert microscope suppl ed with a m cro me

Results

A Morphology

In the functioning rat kidney in situ all the tubules are wide open and distended by tubular fluid and the lining circular cellular wall of the proximal tubules consists of

TABLE I Changes of proximal tubular cellular area as a measure of changes in cellular volume in rat kidney cortex frozen at various states after interruption of blood circulation

	Cellular area of cross sectioned proximal tubules
Functioning state	mean ± 2 s d 667 ± 48 μ^2
After occlusion (45 sec)	1,025 ± 55 μ^2
Incubated cortex slice	1,220 μ^2

flattened, compact cells with an intact luminal brush border, as seen in histological sections of kidneys frozen within 1 sec from cutting the renal pedicle (Fig 1), or by direct inspection through a stereo-microscope of the illuminated kidney surface in situ of the living animal A most rapid change from this appearance is caused by interruption of the renal circulation and filtration In about 20 sec the lumen has disappeared from the proximal tubules, which are all occluded During the occlusion period the cellular volume is maintained unchanged (Leyssac 1964), but a cellular swelling of about 50 per cent has occurred 25—30 sec after clamping of the pedicle (Table I) No further swelling was observed even if kidneys were frozen not until several minutes after removal If, however, a cortical slice was suspended in oxygenated Ringer solutions and incubated for 15 min at 37° C a further swelling had taken place The incubated proximal cells have a very poor and abnormal appearance (Fig 2) compared with that observed in vivo Also the water content has changed markedly, as indicated in the present investigation by the cellular swelling

B Electrolytes

From the activity in slices incubated under aerobic conditions for 15 min at 37 C in Ringers of known specific activity of sodium the concentration of exchangeable sodium was found to be about 100 μeqv/g slice To get an idea about the sodium concentration within the swollen, occluded tubules the histological picture may be used for evaluation of the order of magnitude of the intertubular space" which is histologically defined in contrast to a true extracellular space' The intertubular space represents such a small fraction of the total area of the slice (Fig 2) that an upper limit of 25 per cent extratubular space (including medium adhering to the surface[1]) would seem to be a safe upper estimate Consequently the average tubular sodium concentration is not less than 85—90 μeqv Na/g cells

Analysis of the alkali metal ion content in frozen functioning rat renal cortex are given in Table II and used for a rough estimation of the intracellular concentrations The kidney weight from rats of a body weight of about 250 g was about 0 8 g From the known number of nephrons the length and the luminal radius of the proximal tubules the amount of tubular fluid present in the functioning proximal tubules was calculated to be about 0 18 ml per kidney (Leyssac 1963 a Since the cortical tissue amounts to

[1] By dipping slices in heparinized blood and determining the amount of hemoglobin adhering to the surface after gentle blotting in the usual way an amount of blood corresponding to less than 6 per cent of the tissue weight was found to be adherent to the slice surface (4 expts.)

TABLE II Result of analyses of sodium and potassium from serum and corresponding snap-frozen cortex of rat kidneys Calculations are given in the text

Exp No	Serum μEq/ml Na	K	Total cortex μEq/g wet wt Na	K	Calculated ion content in TV+PI μEq/g cortex Na	K	Calculated intra cellular ion content μEq/g cell Na	K
1	143	4 5	60 4	65 4	58 6	1 72	3 1	108
2	155	4 2	64 6	61 6	63 5	1 72	1 9	102
3	143	5 2	55 0	66 4	58 5	2 14	0	109
4	150	4 0	68 4	60 5	61 5	1 64	11 7	100

about 80 per cent of the total kidney weight in rats, about 0 28 ml of proximal tubular fluid is present per g functioning cortex In 4 experiments the cortical plasma space in the functioning state was found to be 10 6—11 9—13 3—13 1 per cent by the use of labelled serum albumin (^{131}J) From the assumption that the proximal luminal fluid and the interstitial plasma cation concentrations in the functioning state are equal to those in the periferal plasma and *disregarding the small and variable contribution* of distal tubular fluid, the total extracellular sodium and potassium content per g cortex was calculated From these values the "true" intracellular concentrations in the functioning cells were calculated (Table II) As apparent from the table these concentrations are very far from the 'tubular" concentrations found after incubation of slices, but very similar to values given for muscle cells, nerve tissue and red cells

C Na-efflux

When ^{22}Na-loaded slices were flushed with inactive media and the logarithm of the directly recorded activity remaining in the slice was plotted against time an evenly curved line was invariably obtained until completion of the eluation of activity The slopes at any particular efflux fraction varied a little from one slice to the other, but even when the same slice was re incubated and re-eluated some variation in the curves was observed In Fig 3 (A) it is shown that the efflux curve from a slice flushed with isotonic saline is indistinguishable from that of the same re incubated slice when flushed with a Ringer solution containing K^+, Ca^{++}, and Mg^{++} The variation in the slopes of the two curves did not differ significantly from variations observed when a slice was re-incubated and flushed several times with Ringer solution only

Apparently no effect on the efflux could be observed when 2—4-dinitrophenol (DNP) was added to the flushing Ringer in concentrations from 10^{-7} to 10^{-4} M (Fig 3, B), nor did the addition of Oubain 10^{-4} M nor α ketoglutarate influence the slope of the curve Identical efflux curves were also invariably recorded whether angiotensin in concentrations up to 10^{-7} M (10^{-4} g/l) was present or not (Fig 3 C) It should be emphasized that a maximum inhibition of the proximal reabsorption rate was ob-

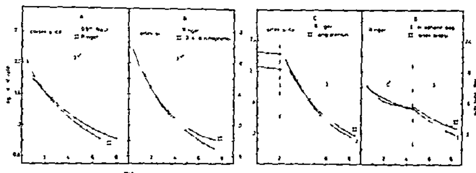


Fig 3 The efflux of sodium from thin cortex slices flushed with different media, from small cubes of cortex, and from a purely passive model. The two curves (I and II) in A, B, and C give the efflux after incubation and re-incubation of the same slice in radioactive medium. The ordinate gives the logarithm of directly recorded activity remaining in the slice (Log Q_t) during the wash-out period as a function of time (abscissa)

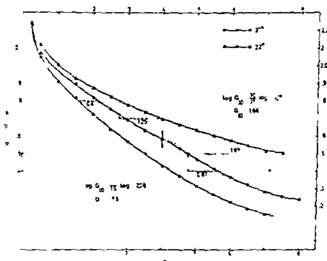


Fig 4 The effect of temperature on sodium efflux from a thin cortex slice flushed with Ringer solution. The three curves are recorded from the same slice after incubation and re-incubation in radioactive medium. The ordinate gives the logarithm of directly recorded activity remaining in the slice (Log Q_t) as a function of time (abscissa)

tained in vivo by i. v. injection to the whole animal of only 25 μl (equal to 25 ng) of this solution (Leyssac 1963 b

The effect of changes in temperature on the efflux rate of sodium is demonstrated in the figures (Fig 3 C and D Fig 4. A great change in the slope of the curve in the case of thin slices is seen when the temperature is changed from zero to 37 C (Fig 3 C). Repeated efflux curves were recorded from a similar thin slice at 22 and 37 C which allows a comparison between the efflux rates at two less differing temperatures during the entire efflux period at comparable fractions of the curve (Fig 4). The efflux curves

TABLE III Uptake of labelled sodium and sulfate after different periods of incubation in Ringer solutions containing 140 mM Na and 6 mM SO_4 Uptake is expressed as activity in per cent of activity in 10 μl incubation medium The ratio gives the volume of distribution of sulfate relative to that of sodium

Incubation period min	c/m in % of 10 μl incub medium		Ratio SO_4/Na
	Uptake sodium	Uptake sulfate	
12	51 0	54 4	1 06
12	67 0	72 0	1 08
12	37 3	37 0	1 00
24	56 0	56 0	1 00
24	54 0	57 0	0 95

and the temperature effect on small cubes ("biopsies") of cortex (about 1 0—1 5 mm thickness) and of a purely passive model (cellophane bags) of the same size and shape are given for the sake of comparison (Fig 3D)

D Uptake of sulfate

When thin cortical slices were incubated under aerobic conditions for different periods of time in Ringer solutions in the presence of tracer amounts of ^{22}Na and $^{35}SO_4$ it is seen from table III that after 15 min or more sulfate, in concentrations of 6—10 mM was taken up in a space equal to the sodium space If, however, the concentration of sulfate in the incubation medium was decreased by a factor 10 preliminary experiments showed that sulfate was accumulated in the slices, indicated by a sulfate — space exceeding that of water by a factor of 2

E Efflux of sulfate

Fig 5 shows that the eluation curves for sulfate and sodium when recorded simultaneously from the same slice, were closely parallel during almost the entire course of the eluation in slices incubated in Ringer solutions at a sulfate concentration of 6—10 mM

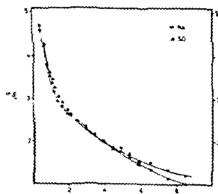


Fig 5 The simultaneous efflux rate of sodium and sulfate from thin cortex slice The Ordinate The logarithm of c m in wash out medium collected over 5 sec $\left(\text{Log}\,\frac{10}{t}\right)$ Abscissa time

Discussion

It was shown by Hanssen (1960) that the process of transcellular transport continues in the early post mortem period with the consequence that the proximal tubular lumina had disappeared within 30—40 sec Later investigations in rats by Steinhausen *et al* (1963) and by Leyssac (1963 a) have further established that the proximal tubular lumina in rats are completely occluded in the convoluted part of the proximal tubules within 20 sec after the interruption of the renal circulation and filtration This fact would mean that no transcellular transport can apparently take place from the lumen side of the proximal cells in isolated renal cortical tissue unless the lumina are restored The present investigation demonstrates that this is not the case using incubated cortical slices The incubated kidney cortical slice is therefore apparently no model for studies on the process of transcellular transport

The present results of calculated intracellular cation composition in functioning proximal tubular cells (Table II) disclosed a very high potassium, low sodium concentration similar to values obtained in nerve tissue, muscle cells and red cells

From the very moment of luminal occlusion following interruption of blood circulation and filtration a series of events are initiated, leading finally to the completely abnormal ion composition — and distribution in the incubated slice It was observed in a previous study (Leyssac 1964) that a swelling of about 50 per cent occurred in the proximal cells already immediately following the luminal occlusion (20—25 sec after interruption of circulation) This early swelling which suggests a marked change of the intracellular ion composition and water content, inferred to us that water and sodium leaked into the cells from the interstitial space The changes in ion composition of freshly cut slices immersed in Ringer solution under aerobic conditions at 37° C was studies thoroughly by Whittam and Davies (1953) A further and rapid change in electrolyte composition occurred Initially sodium was gained and potassium was lost from the slice Although this process was partly reversed in the subsequent period the total cation and water content was increased when a steady state was reached after 15—20 min of incubation mostly because of a net gain in sodium So this is the steady state condition of an incubated cortical slice at 37° C The net gain in sodium and water during incubation is consistent with further swelling observed in the present investigation following incubation In close agreement with these observations an "average intracellular" or more correctly average tubular concentration of sodium of about and not less than 85—90 μeq/g cells was estimated in the occluded tubules of incubated slices (p 5)

Although the incubated kidney slices are greatly swollen at 37° C with a cellular content of water and sodium incomparable with that of physiological conditions it still remains a fact that slices may maintain some cation concentration differences between the tissue as a whole and the incubation medium and that these modest differences are related to the metabolism of the slice However it remains an unsolved problem whether these concentration differences exist between the medium and *each cell in a* rather uniform pool of functioning cells An alternative interpretation of the cation distribution within the slices would be it appears a two-pool system consisting of a rather small pool of tubules with normal and functioning cells and a large pool of tubules in which all the cells are more or less disintegrated and with respect to sodium in diffusion equilibrium with the medium The total tissue water would then consist of two components of which a major component of 75—80 per cent contains 98 per cent

of the total sodium pool at a concentration of 140 meq/l, whereas the minor component of 20—25 per cent of the water contains only about 2 per cent of the sodium at a concentration of about 10 meq/l cellular water

From the efflux curves it is apparent that the rate of sodium loss from the incubated slices was unaffected by addition to the flushing medium of known inhibitors of active sodium transport, and curves not significantly different from each other were obtained no matter whether the medium contained K^+, Ca^{++}, and Mg^{++} or not. Thus, any "active" component in the sodium efflux could not be qualitatively demonstrated by this technique

When changing the temperature in the medium from zero to 37° C a marked change in the slope of the efflux curve was observed in the thin slices, which might at first glance erroneously suggest an "active" component in the sodium efflux. If, however, the sodium activity is exchanged by a one-dimensional diffusion process in a tissue sheet of non-uniform thickness, like that of a tissue slice cut by free hand, each thickness will represent one single pool The recorded efflux curve is then a composite curve of a serie of logarithmic slopes each characterizing one single pool with one time constant ($Ae^{-\alpha t} - Be^{-\beta t}$), the significant number of single pools (A, B, etc) at any particular time depending upon the time constants (α, β, etc) A simple comparison between slopes of tangents then has no clear physical meaning Thus, if calculated from the terminal slope at zero° C and the slope after flushing for 1 min at 37° C the apparent "Q_{10}", which ranged between 2 1 and 2 3, may be characterized as a "false Q_{10}." The efflux curve may, however, still be used for an approximation of the temperature coefficient of the process, since the significant number of exponential terms will become reasonably comparable provided the time constants do not differ too much This situation may be approached by narrowing the temperature interval Thus, when a thin slice was eluated and re-eluated at two less differing temperatures (22° and 37° C) or when the temperature was shifted between these two temperatures late in the wash-out period, it was possible to estimate the effect corresponding either to identical abscissa values (time) or to identical ordinate values (activity), which gave similar results As apparent from the figure (Fig 4) these somewhat more "meaningful" Q_{10} values were considerably lower, and furthermore similar values were obtained early and late in the washout period The effect of changes in temperature was also studied in a purely passive model, small bags of cellophane filled with colloid free Ringer solution "Q_{10}" ranged from 1 22 to 1 31, in accordance with the expected value for free diffusion of sodium in water If, however, albumin was added to the Ringer inside the bags, which may be considered then a more appropriate model, "Q_{10}" values indistinguishable from those obtained from cortical tissue were found

The data on the effect of temperature, thus, are compatible with the suggestion that sodium within the cortical slice is passively exchanged with that of the medium

The finding that sulfate during incubation in a Ringer medium containing 0 6 mM sulfate was accumulated in the slice is consistant with the findings of Deyrup and Ussing (1955) at low concentrations of sulfate However, at concentrations in the medium of sulfate of 6 mM or more this anion was distributed in a space equal to the size of the sodium space This finding would seem unexpected if the cellular integrities were conserved with almost normal permeability properties of the cells It might still be postulated that sodium and sulfate were distributed in different subcellular compartments. The simultaneously recorded efflux rate of sulfate and sodium fr

however that the fractional rate of loss of activity per un

Discussion

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Although the incubated kidney slices are greatly swollen at 37° C with a cellular content of water and sodium incomparable with that of physiological conditions it still remains a fact that slices may maintain some cation concentration differences between the tissue as a whole and the incubation medium, and that these modest differences are related to the metabolism of the slice However, it remains an unsolved problem whether these concentration differences exist between the medium and each cell in a rather uniform pool of functioning cells An alternative interpretation of the cation distribution within the slices would be it appears a two-pool system consisting of a rather small pool of tubules with normal and functioning cells and a large pool of tubules in which all the cells are more or less disintegrated and, with respect to sodium in diffusion equilibrium with the medium The total tissue water would then consist of two components of which a major component of 75—80 per cent contains 98 per cent

the fraction of "truely" intracellular sodium in cortical slices In accordance with Whittembury's data a quantitative analysis (Bojesen, Leyssac and Svejgaard Nielsen 1965, subsequent paper) has revealed that as much as 15—20 per cent of the exchangeable sodium pool in about 40 per cent of the tissue water is not in diffusion equilibrium with the medium but is lost at a more slow rate

The present calculated very low sodium — and high potassium concentrations in the cytoplasm of functioning proximal tubular cell

cytoplasmatic

basement membrane to the outside From the concentration difference of sodium ($\frac{c_o}{c_i} = \frac{140}{14}$, table II) and a potential difference across this membrane of 74 mV (Whittembury 1960, Giebisch 1961, Whittembury, Sugino and Solomon 1961), which was some 10 mV less than the potential difference calculated from our observed potassium concentration ratio the minimal work, as calculated from the equation

$$w = RT\ ln \left(\frac{c_o}{c_i}\right) + EF \text{ (Levi and Ussing 1948)}$$

will be 3 200 cal per eqv of Na (37° C) Since 7 eqv Na is found to be each suprabasal eqv of and Thaysen 1961) with become 80—90 per cent ably high (the calculation rests upon the preassumption of no resistance in the membrane), and therefore throw considerable doubt on the general assumption underlying the calculation An alternative possibility is that sodium transported across the cellular wall is not mixed with the cytoplasmatic pool but is on the contrary confined to special intracellular structures allowing a localized high sodium concentration Accordingly the transcellular transport process includes cytophysiological mechan finding of a

present results an incubated mammalian kidney cortex slice at 37 C may be visualized as a piece of tissue composed largely of two tubules non of which with the

the cell

turnover rate of sodium with that of intracellular sodium of other tissues such as striated muscles

The F H well scillation counter and scaler were generously supplied by Grants from the Tuborg Fond and support to this work was given to one of us (P P L) by Grants from Statens almindelige Videnskabsfond and Novo Fonden for which the author wishes to express his gratitude

[1] If the calculation is made from the assumption of an intracellular concentration of 30 μeq Na g water the efficiency will still become unreasonably large (70—80 per cent

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The Antagonistic Effect of Human Plasma Albumin on the Insulin Stimulated Glucose Uptake of the Isolated Rat Diaphragm

By

JAK JERVELL

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Abstract

Jervell J *The antagonistic effect of human plasma albumin on the insulin stimulated of glucose uptake of the isolated rat diaphragm* Acta physiol scand 1965 65 33—44 — Albumin isolated by several different procedures antagonized the effect of insulin on the glucose uptake of the isolated rat hemidiaphragm The antagonism was still present after the pH reducing effect of the albumins had been corrected A decrease in pH was also found to have some insulin antagonistic effect There was no correlation between the antagonistic effect and the free fatty acid content of the albumins and adding palmitate to a nonantagonistic albumin did not increase its antagonistic activity The antagonistic effect cannot be explained by the calcium and magnesium binding properties of the albumin

An insulin antagonistic effect of human plasma albumin on the glucose uptake of the isolated rat hemidiaphragm was first observed by Vallance-Owen *et al* (1958a) Lowy *et al* (1961) confirmed this and in addition observed that no antagonism could be demonstrated on the epididymal fat pad of the rat The amount of antagonistic activity is higher in albumin from diabetics and prediabetics than from normal subjects (Vallance Owen and Lilley 1961) and also in a high proportion of patients with cardiac infarction (Vallance Owen and Ashton 1963a)

Alp and Recant (1964) besides verifying Vallance-Owen s observation that diabetic albumin will antagonize the insulin effect at a lower concentration than normal albumin also report further experiments on adipose tissue In this tissue they found no antagonism but instead a marked insulin like activity (ILA) of their albumin This ILA is probably caused by insulin itself since it is supressible by anti insulin serum

Keen (1963) and later Cameron Keen and Menzinger (1964 have not found any antagonistic effect in their albumin preparations even though these were extracted by the same thricloroacetic acid — ethanol procedure used by other workers Their albumin gave a marked ILA not only on fatty tissue but also on the diaphragm

Buse and Buse (1964) using commercial Cohn fractionated albumin, found insulin antagonistic activity on several insulin sensitive reactions in the diaphragm preparation They explain this antagonism partly by the increase in hydrogen ion concentration of the incubation media caused by the albumin The remaining effect they suggest may be caused by the free fatty acid content of the albumin

The present study was initiated to investigate the effect of albumin on various insulin-sensitive metabolic processes in the rat diaphragm In view of the conflicting reports which exist, it was neccessary to ascertain the presence of an insulinantagonistic activity, and to evaluate its dependence upon the pH lowering effect, free fatty acid content and calcium and magnesium binding property of the plasma albumin

Methods and material

Albumin

Human albumin was extracted from fresh, heparinized plasma of normal subjects or from stored plasma from outdated bank blood Several procedures were used

I The trichloroacetic acid (TCA) ethanol procedure as used by Vallance Owen *et al* (1958) and most other workers in this field

During dialysis of the TCA-ethanol albumin mixture a slight precipitate was formed which was partly removed by centrifugation either before freeze-drying or after the albumin was redissolved for testing

II Plasma was treated with ten volumes of acid (HCl) methanol as described by Michael (1962) After centrifugation and filtration the albumin was precipitated by neutralization collected and washed twice with methanol and twice with ether After the albumin had been air dried, the complete removal of the ether was assured by re dissolving in water and freeze-drying

III Ammonium sulphate fractionation (Kendall 1941) The fraction precipitated between 60 and 75 per cent saturation was collected by filtration dissolved in water and extensively dialysed before freeze drying In some cases the supernatant after 60 per cent saturation was dialysed directly

IV Starch block electrophoresis in veronal buffer (ionic strength 0 1 pH 8 6 Bloemendal 1960) The albumin fraction was cut out eluted with d stilled water in a Buchner funnel dialysed and freeze-dried

Cystalline human albumin from Nutritional Biochemical Corporation (V) and various batches from A/B Kabi (VI) were also used

All dialysis was carried out in Visking tubing 20/32 inches for 72 hrs at room temperature (I) or at 2 C (III and IV)

The identity of the albumins was checked by paper electrophoretic analyses in veronal buffer (pH 8 6 ionic strength 0 1) Albumin produced by method I was electrophoretically homogenous whereas slight globulin contaminations were found in albumins prepared by methods II and III and in the commercial preparations

The free fatty acid (FFA) content of the albumins was determined according to Dole (1956) using a 5 per cent solution of albumin in Krebs Ringer bicarbonate buffer (Umbreit Burris and Stauffer 1954)

Incubation Technique

Male or female albino rats of a local strain weighing 120—160 g were used in any one experimental day the rats were of the same sex and weight ($\pm$ 5 per cent) The rats were fed a standard diet (21 per cent protein 3 per cent fat and about 56 per cent carbohydrate) and fasted 20 hrs before use

Krebs Ringer bicarbonate buffer (Umbreit, Burris and Stauffer 1957) was used Insulin was added by serial dilution from a stock solution containing 50 units per ml in 0 6 per cent acetic acid. Glucose and albumin were added in appropriate amounts

Only the lateral and anterior parts of the rat hemidiaphragms were used. After 14—20 min soaking in cold Krebs Ringer buffer (4—10 C) the hemidiaphragms were blotted gently on filter paper and placed in 1 ml incubation medium in 5 ml Erlenmeyer flasks These were closed with rubber caps and gassed for 5 min through injection needles with a gas mixture of 5 per cent carbon dioxide in oxygen The flasks were incubated for 90 min at 37° C and shaken at a rate of 110 double oscillations per minute The tissue samples were then rinsed in water and dried at 105—110° C for 2 hrs and weighed The glucose concentration of the incubation media was determined in triplicate before and after incubation by the glucose oxidase method (Hugget and Nixon 1957)

The initial glucose concentration in the media was 3 mg per ml after incubation it had fallen to around 2 5 mg per ml in the absence of insulin and to between 1 5 and 2 mg per ml in the presence of 1 000 microunits of insulin per ml. The glucose uptake was claculated as mg glucose taken up per gram dry tissue per 90 min

The pH of the incubation media was determined with a glass electrode at 37° C in an atmosphere of 95 % O_2 — 5 % CO_2 Adjustment of pH was made with 0 154 M sodium b carbonate

In some experiments palmitate was added to the albumin-containing media This was done by adding 5 µl per ml of warm (70° C) 0 154 M sodium palmitate to the incubation medium at 37 C before adjusting the pH

Usually 6 or 9 rats were used in one experiment giving 12 or 18 flasks with one hemidiaphragm in each Three hemidiaphragms were incubated in identical media thus 2 or 4 different incubation mixtures could be tested besides the standard Krebs-Ringer buffer ± insulin The hemidiaphragms from the same rat were not paired " (Keen 1963) but all hemidiaphragms were distributed randomly between the different incubation media making it possible to compare the glucose uptakes under different cond tions directly By rigorous control of experimental conditions the described procedure gave close enough parallels to demonstrate significant antagonism of the insulin effect In most cases however the same variables were tested on two or more different days giving at least 6 parallels for each mean The insulin effect was invariably h ghly significant and in the order of 60 to 130 per cent of basal glucose uptake Statistical analysis of the results was carried out and Student s t test used for determining the significance of differences between glucose uptakes under various conditions (Croxton 1959)

The coefficient of correlation was calculated by the Pearson product moment formula (Croxton 1959)

Materials

Crystall ne bovine insulin was obtained from Allen & Hanburys Ltd and the British Drug Houses Ltd batch no 2266 23 6 units per mg and from Novo Industri A S lot no 5467 10 crystallized the activity of the latter was assumed to be 23 6 units per mg Otherwise standard h gh purity chemical and biochemical reagents were used throughout

Results

Albumin isolated by all the methods described antagonized the effect of insulin on the glucose uptake of the cut hemidiaphragm (Table I The antagonistic effect was very variable this might be due to varying pH lowering effects of the different albumins as no checking or adjusting of the pH of the incubation media was carried out in these experiments The variable effects may also partly be due to differences in the amount of antagonism extracted by the different methods and to differences in the amount of antagonism present in the original plasma from which the extraction was made A stimulatory effect of the albumins on the basal glucose uptake was only rarely found expts 1 2 and 12 This is in contrast to the results of Cameron *et al* 1964 who found that their TCA-ethanol extracted albumins always exhibited a stimulatory effect on basal glucose uptake

TABLE I Glucose uptake of cut hemidiaphragms, incubated in buffer with and without albumin in the absence and presence of insulin

Exp no	Method	Glucose uptake of muscle (mg per g dry muscle per 90 min) a Buffer alone	b Buffer + albumin (5 per cent)	c Buffer + insulin (10^{-2} units per ml)	d Buffer + albumin + insulin	Per cent inhibition caused by albumin $100\frac{(c-d)}{(c-a)}$
1	I	24.5±2.0 (3)	34.0±0.3 (3)	47.3±1.5 (3)	33.6±0.9 (3)	60
2	I	30.3±1.9 (3)	44.4±1.2 (3)	66.8±2.3 (3)	44.7±2.3 (3)	61
3	I+IV	24.8±1.2 (3)		43.7±3.9 (3)	25.1±2.4 (3)	98
4	I	24.4±4.2 (3)	28.9±3.4 (3)	36.6±2.0 (3)	29.7±0.8 (3)	57
5	I	34.6±4.5 (3)	30.6±3.2 (3)	60.0±0.5 (3)	42.9±2.6 (3)	67
6	I	26.4±1.0 (3)	26.0±2.1 (3)	54.2±1.3 (3)	43.4±2.3 (3)	38.5
7	II	24.4±0.7 (3)	26.0±0.5 (3)	46.6±3.2 (3)	25.3±0.8 (3)	96
8	II	26.4±1.0 (3)	23.3±2.1 (3)	54.4±1.3 (3)	29.4±1.2 (3)	89
9	II	27.1±2.0 (6)	30.7±1.5 (6)	63.0±2.9 (6)	31.9±2.3 (6)	87
10	III	37.0±1.9 (4)	35.7±1.9 (4)	57.9±1.2 (4)	42.6±3.8 (4)	73
11	IV	28.7±2.6 (3)		51.5±5.1 (3)	36.9±0.6 (3)	64
12	V	28.5±1.1 (4)	34.1±0.6 (4)	53.3±2.2 (4)	47.1±0.8 (4)	25
13	VI	28.0±1.6 (3)	28.9±2.3 (3)	50.0±1.8 (3)	46.9±3.8 (3)	14
14	VI	26.4±1.9 (3)	28.7±1.5 (3)	55.3±1.7 (3)	40.4±2.8 (3)	51.5

In this and the following tables ± standard error of the mean and number of observations are given with each figure. Roman numerals refer to method of albumin isolation described in the text. In exp. 3 the albumin concentration was 3.8%. The difference between a and b is significant ($p < 0.01$) in exp. 1, 2 and 12. The difference between c and d (insulin antagonism) is significant in all experiments except no. 13. (In exp. 4, 11 and 12 $p < 0.05$, in the remaining exps $p < 0.01$.)

In exp. 3 the albumin used in exp. 2 was subjected to starch block electrophoresis before testing.
No adjustment of pH was performed in these experiments.

The albumin used in exp. 2 was subjected to starch block electrophoresis and retested (exp. 3). It was then found to antagonize the insulin effect completely and presumably the stimulatory effect on basal glucose uptake was also removed by this treatment.

pH

The Krebs-Ringer buffer at 37° C in an atmosphere of 5% CO_2—95% O_2 had a mean pH of 7.44 ± 0.026 (± S.D.) 13 measurements on 13 different buffers). Solutions of 7 different albumins (5 per cent in Krebs-Ringer bicarbonate buffer) prepared by method I had a mean pH of 7.07 ± 0.08 and similarly 2 albumins produced by method III gave pH values of 7.15 and 7.23. Kabi albumin RfK 23 gave a pH of 6.85 and Kabi albumin RdO 22 gave 7.20.

TABLE II Glucose uptake of cut hemidiaphragms at different pH values

	Glucose uptake in mg per gram tissue per 90 min	
	No insulin	Insulin 10^{-3} units per ml
pH 7.4	27.5 ± 1.3 (6)	58.6 ± 1.5 (6)
pH 7.25	*23.8 ± 0.7 (6)	**48.1 ± 1.9 (6)
pH 6.97	*22.9 ± 1.1 (6)	**35.6 ± 1.9 (6)

The glucose uptake at the two lower pH values is significantly (*$p < 0.05$, **$p < 0.005$) lower than the uptake at pH 7.4. The pH of the *media* was adjusted by substituting some NaCl for $NaHCO_3$ in the Krebs Ringer buffer.

TABLE III The effect of various albumin preparations on glucose uptake of cut hemidiaphragms at *adjusted* pH and at pH 7.45

		Glucose uptake in mg per gram dry tissue per 90 min			
Additions	pH of unadjusted medium	No insulin pH unadjusted	10^{-3} units of insulin per ml pH unadjusted	10^{-3} units of insulin per ml pH adjusted to 7.45	FFA content of medium µeq per ml
None	7.45	*23.8 ± 0.9 (12)	52.1 ± 1.4 (14)	51.1 ± 2.0 (9)	0
5% albumin Method I	7.35	24.0 ± 2.4 (3)	*45.5 ± 2.0 (9)	*43.0 ± 1.8 (9)	1.3
5% albumin Method III	7.23	23.8 ± 3.6 (3)	*47.9 ± 2.0 (8)	*44.3 ± 2.0 (9)	0.7
5% albumin KABI Rd8 22	7.20	*20.1 ± 1.3 (3)	**46.5 ± 1.4 (12)	47.4 ± 1.3 (9)	1.0
5% albumin KABI Rfk 23	6.85		**34.7 ± 1.4 (9)	**43.1 ± 1.3 (9)	1.2
5% albumin Method III	7.15	21.6 ± 1.2 (6)	**39.7 ± 1.2 (11)	48.8 ± 1.7 (6)	0.4

Uptakes marked with an asterisk* differ significantly ($p < 0.05$) from uptake without albumin (upper line). 2 asterisks** $p < 0.01$. The pH was adjusted by adding 0.154 M $NaHCO_3$ to the media. The albumin isolated by method I was adjusted to pH 7.4 before freeze

TABLE IV Effect of 5% albumin on glucose uptake of cut diaphragm after adjusting pH to 7 45

	Glucose uptake in mg per gram tissue		
Exp no	a Buffer alone	b Buffer + 5% albumin	c Buffer + insulin (10 ⁴ units per ml
1	27 8±2 6	32 2±2 3	59 7±4 1
2	24 5±2 6	26 0±1 5	51 3±1 1
3	28 3±0 8	32 2±1 7	50 9±2 (
4	20 9±0 4	22 4±2 2	53 8±2
5	28 8±5 0	37 3±1 7	58 4±3
6	24 9±1 4	31 7±2 4	57 2±1 6
7	28 7±1 1	35 4±1 4	56 6±2 4
Mean	25 7±0 9 (21)	31 0±1 2 (21)	55 4±1 0 (21)

The albumins were all prepared by the TCA-ethanol method
The number of observations is 3 for each figure except for the lower line which is the mean of
Difference between columns c and d (insulin antagonism) is significant ($p < 0\ 05$) in exp
In the lower line (mean) the antagonism is highly significant ($p < 0\ 001$)

It was then of interest to study the influence of pH changes on the glucose uptake of the rat hemidiaphragm The results of this experiment (Table II) show that lowering the pH to 7 25 caused a moderate decrease in both basal and insulin stimulated uptake at pH 6 97 the insulin-stimulated uptake showed further marked decrease

When the pH of the albumin-containing incubation media was adjusted to 7 45 the insulin antagonistic effect was reduced in those albumins which had the most marked pH lowering effect (Table III Most of the albumins however retained a significant insulin antagonistic effect after the pH adjustment

Table IV similarly shows the insulin antagonistic effect of 7 different TCA ethanol extracted albumins To avoid the effect of the variable pH the incubation media were adjusted to pH 7 45 before testing All the preparations were insulin antagonistic the insulin effect was 22—48 per cent lower (in one case 10 per cent) in the presence of albumin For the mean of all these experiments the insulin antagonistic effect is 28 per cent and highly significant p 0 001

During incubations the pH of the incubation media fell from 7 45 to around 7 30 the presence of albumin made no difference in this respect

Free fatty acids

Table V shows the effect of adding palmitate to the albumin containing medium In this experiment the Kabi albumin RdO 22 which is not antagonistic see Table III) was used It is seen that the glucose uptake or insulin effect was not influenced by the addition of palmitate

d Buffer + albumin + insulin	Ins effect in absence of albumin	Inhibition caused by albumin	% inhibition caused by albumin	FFA conc of medium µeq per ml
44 5±2 8	31 9	15 2	48%	1 42
45 3±1 5	26 8	6 0	22%	1 90
45 6±1 2	22 6	5 3	23%	0 89
46 7±2 9	32 9	7 1	22%	1 16
46 7±2 6	29 6	11 7	40%	1 82
46 2±1 4	32 3	11 0	34%	0 87
53 8±2 5	27 9	2 8	10%	0 82
46 9±1 0 (21)	29 4	8.5	29%	1 27

all experiments
1 2 5 and 6

TABLE V Effect of adding palmitate to albumin-containing media on glucose uptake of cut hemidiaphragms

	Glucose uptake in mg per gram dry muscle	
	No insulin	Insulin 10 ³ units per ml
Buffer alone No FFA	27 2±1 8 (6)	49 6±1 2 (6)
Buffer + 5% albumin (Rd0 22 KABI) FFA conc 1 µeq/ml	26 7±2 (6)	47 9±1 9 (6)
Buffer 5% albumin (Rd0 22 KABI) added palmitate FFA conc 1 8 µeq/ml	30 0±1 2 (6)	49 1±1 9 (6)

The insulin effects are highly significant (p 0 001) but there is no significant inhibition of glucose uptake when FFA are present
The pH of all incubation media was adjusted to 7 45

The FFA content has been determined in several of the albumins used the results are given Table III and IV There is clearly no correlation between FFA content and antagonistic effect (the coefficient of correlation between FFA concentration and insulin antagonistic effect in mg glucose per gram dry tissue per 90 minutes is 0 13)

TABLE VI The effect of varying the calcium and magnesium concentrations on glucose uptake of cut hemidiaphragms

	Glucose uptake in mg per gram dry weight per min	
	Buffer alone	Buffer + insulin 10^{-1} units per ml
Krebs Ringer buffer		
5 07 meq Ca^{++}/l	27 9±1 7	57 2±2 1
2 39 meq Mg^{++}/l	(6)	(6)
2 54 meq Ca^{++}/l	33 8*±1 9	54 7±2 7
1 20 meq Mg^{++}/l	(6)	(6)
1 28 meq Ca^{++}/l	33 1±3 1	54 1±2 5
0 60 meq Mg^{++}/l	(6)	(6)

The uptake marked with an asterisk* is significantly higher ($p < 0\ 05$) than uptake in the normal buffer Differences in presence of insulin are not significant ($p > 0\ 40$)

Calcium and magnesium

The Krebs Ringer buffer used contained 5 07 meq calcium and 2 39 meq magnesium per liter Analysis of the upper albumin free phase after ultracentrifugation showed that about 50 per cent of these ions were bound to the albumin when this was present in a concentration of 5 per cent It therefore seemed important to examine the effect of reducing the calcium and magnesium concentrations in the incubation medium The results of these experiments are shown in table VI, and it is seen that there was a slight stimulation of marginal significance of basal glucose uptake at the lower concentrations, while the insulin stimulated uptake was not affected

Discussion

It is not surprising that albumin, dialysed against distilled water and subsequently freeze-dried, should decrease the pH of a bicarbonate buffer of pH 7 45 Albumin is ... buffer capacity and at its isoionic point ... ffect is to be ... eeze-drying (See Table III)

The effect of changing pH on the insulin stimulated glycogen formation of the cut rat hemidiaphragm has been studied by Stadie and Zapp (1947) Using a phosphate buffer they found no change in the insulin effect on glycogen formation when the pH was varied between 6 8 and 7 4

Similar studies in bicarbonate buffered media seem to be lacking Bhattacharya (1961) in his thorough study of the effect of metal ions on glucose uptake, does not make any ... tables ... may be inferred ... nd

From the results in the present study it seems clear that increased hydrogen ion concentration in a bicarbonate buffer, although it is associated with a decrease in the basal glucose uptake has a much greater effect on the insulin stimulated glucose uptake, in other words increased hydrogen ion concentration has an insulin antagonistic effect However, even after the pH of the albumin-containing media had been adjusted to the pH of the Krebs Ringer buffer, a significant insulin antagonistic effect remained

It seemed possible, as also suggested by Buse and Buse (1964), that FFA bound to albumin might account for this effect It has been shown in the perfused rat heart (Shipp et al 1961) that 0 4 mM FFA will reduce the oxidation of glucose markedly and the glucose uptake slightly, while the glycogen content is increased They suggest that this might explain the finding of high glycogen levels in heart muscle as opposed to liver and diaphragm during fasting and diabetes mellitus Similar results have been obtained by Randle et al (1964), and Bowman (1962) On the rat diaphragm the same type of effect was observed by Buhring (1963) He used a special FFA free albumin and added specific amounts of known fatty acids, he found that when the FFA concentration was increased from 2 25 to 10 μmoles per ml the glucose oxidation, ^{14}C-glucose incorporation into glycogen and glucose uptake were depressed Even the lowest of these FFA concentrations however was well above any found in the present study Randle et al (1963) reported that 0 6 mM palmitate inhibited the insulin-stimulated glucose uptake The effect however was small, and no data on statistical significance was given In the present study the FFA content of the albumin-containing incubation media varied from 0 4 to 1 9 μmoles per ml and there was very little correlation (coefficient of correlation 0 13) between FFA concentration and insulin antagonistic effect Adding palmitate to a non antagonistic albumin did not cause any antagonistic effect to appear

Similarly Buse and Buse (1964 personal communication) reported that they could not produce insulin antagonism by adding FFA to nonantagonistic amounts of albumin

Consequently the insulin antagonistic effect of the albumins cannot be explained by their content of free fatty acids

Another well known property of plasma albumin is its ability to bind calcium and magnesium Carr (1955) found that the strength of the binding is about the same for both ions consequently the relative binding of the two ions would be in direct proportion to their concentrations This is in accordance with our findings where about 50 per cent of both calcium and magnesium were bound in a 5 per cent albumin solution It was also found that lowering the calcium and magnesium concentrations to a half or a quarter of those in the Krebs Ringer buffer did not decrease the glucose uptake on the contrary it seemed as though the basal uptake may be increased Bhattacharya (1961) finds that magnesium is necessary for the insulin effect and that calcium counteracts the effects of magnesium. However, the effect of increasing the calcium concentration could be overcome by increased magnesium levels and the Ca/Mg ratio is most important

One is left with the conclusion that serum albumin isolated by several different procedures antagonizes the effect of insulin on the glucose uptake of the rat hemidiaphragm The effect is not explained by the pH reduction caused by albumin nor by its content of FFA or by its calcium and magnesium binding properties

Buse and Buse (1964, personal communication) stated they were able to remove the antagonistic activity from their Cohn fractionated albumin by dialysis Their antagonist therefore cannot be identical with the one found in this study since in both methods

I and III the albumin was dialysed for 72 hrs before freeze-drying In the present study the only non-antagonistic albumin sample was the Kabi albumin RdO 22, isolated by a modified Cohn procedure (Dr Bjorling, Kabi, personal communication) The Cohn method, therefore, seems to yield an albumin of no, or relatively low, antagonistic activity

The TCA-ethanol procedure is the method used by most workers studying the albumin-bound insulin antagonism (Vallance-Owen *et al.* 1958, Lowy *et al* 1961, Keen 1963, Cameron *et al* 1964, Alp and Recant 1964) This method is based on the observation made independently by Delaville, Delaville and Delaville (1954), Levine (1954), and Korner and Debro (1956) that TCA-precipitated plasma albumin dissolves in TCA-ethanol and can be recovered after dialyses by freeze-drying Schwert (1957) showed that after this treatment albumin retains its characteristics with regard to electrophoretic mobility, sedimentation, crystallization, solubility and thermal stability. Ram and Maurer (1958) found that albumin treated in this way was identical with native albumin in its immunochemical character and elimination pattern in the rabbit A slightly increased susceptibility to trypsin was found, this increase, however, was insignificant in comparison with that caused by heat denaturation Debro *et al* (1957) after TCA-ethanol treatment of albumin, found slightly divergent properties by moving boundry electrophoresis at pH 5 4 whereas at pH 8 5 the patterns were identical The TCA-ethanol procedure therefore has many advantages as an albumin extraction procedure, it is relatively simple and yields a product which in most charcteristics, including the immunological, is identical with albumin isolated by more complicated methods

Of all the other proteins tested, Schwert (1957) found that only insulin was soluble in TCA-ethanol after TCA precipitation Plasma-insulin therefore probably follows the albumin into solution in TCA-ethanol One might have expected most of the insulin to be removed during the extensive dialysis which follows, Craig *et al* (1957), however, report widely different permeability of insulin through 'Visking' tubing of different sizes Cameron *et al* (1964) who found that their preparations had a strong insulin like effect both on the rat diaphragm and fat pad, used 'Visking' tubing 18/32, this was reported by Craig to be impenetrable to insulin What types of tubing other workers have used is not known The present study employed 20/32 inches tubing which according to Craig is easily permeable to insulin When insulin is dissolved in TCA-ethanol and dialysed for three days no ILA is found inside the dialysis bags whether 18/32 or 20 32 inches tubing is used (unpublished results) It is possible, however, that insulin behaves differently in the presence of albumin Indeed Keen (1963) found that adding a potent insulin neutralizing plasma inhibited the ILA of his preparations and Alp and Recant (1964) found that on an average 65 % of the serum ILA could be recovered in their TCA-ethanol extracts when tested on the fat pad and this activity could be counteracted by insulin antiserum Their albumins however, completely antagonized the effect of insulin on the diaphragm and showed no ILA on this preparation

The TCA-ethanol procedure therefore extracts albumin, insulin and an insulin antagonist from plasma The different results obtained with this extract by different workers are probably explainable by small differences in the procedure which alter the relative amounts of insulin and insulin-antagonist retained The insulin antagonist is probably bound to albumin since it follows the albumin in several extraction procedures including starch-block electrophoresis but is not identical with albumin since it can be removed on an acetylated cellulose column (Vallance-Owen *et al* 1958 b)

The nature of the antagonist is not known Ensinck and Vallance Owen (1963) have proposed that it is identical with the albumin-bound B-chain of insulin This is as yet unconfirmed, but the suggestion opens many new possibilities regarding the etiology of diabetes mellitus, especially in view of the finding of increased insulin antagonism in the albumin from essential diabetics, prediabetics and many unsymptomatic members of diabetic families (Vallance-Owen and Ashton 1963 b)

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Acute and Chronic Effects of Preoptic Lesions

By

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Abstract

Andersson, B., C. C. Gale, B. Hökfelt and B. Larsson. *Acute and chronic effects of preoptic lesions.* Acta physiol. scand. 1965. *65.* 45—60. — The effects of preoptic and preoptic/hypothalamic lesions were studied in unanesthetized goats. A gradual destruction of the entire preoptic region (the "heat loss center") was induced by proton irradiation in two animals. The lesions caused a permanently raised heat-dissipation threshold, an impaired resistance to cold, a complete adipsia and a lack of thermal inhibition of food intake. A febrile response to pyrogen was obtained in spite of the destruction of the preoptic region. In contrast to the proton lesions a rapidly induced radio-frequency (RF) coagulation was fatal if restricted to the preoptic region. It caused initial strong shivering and peripheral vasoconstriction, sympathico-adrenomedullary and thyroid activation, severe hyperthermia, hyperglycemia, and death within 16 hours in three goats studied. An ability to respond to severe hyperthermia with polypnea remained in these goats. In two goats preoptic RF lesions were extended to involve most of the anterior hypothalamus. These animals survived an initial period of hyperthermia and sympathico-adrenomedullary activation. One of them was studied for 8 months and showed similar disturbances of temperature regulation and alimentation as the proton irradiated goats. In one goat the RF lesions was extended further caudally to involve also the mid hypothalamic region. The immediate effects were virtual disappearance of urinary excretion of noradrenaline, development of marked hypothermia, and considerable hypoglycemia.

The observation of Magoun *et al.* (1938) that local heating of the preoptic region of the cat activates various heat dissipation mechanisms directed attention to this part of the brain as a site for central "warmth detectors". Since then the role of the preoptic region as a "heat loss center" has been further elucidated in many other species by the experimental techniques of local displacement of brain temperature and of electrical stimulation or electrophysiological recordings of neuronal activity (cf. C. von Euler 1961 and Hardy 1961). Ablation experiments offer another possible way to study the importance of the preoptic region in temperature regulation, but reports on the thermoregulatory effects of preoptic lesions are relatively few. The reason may be that acutely induced

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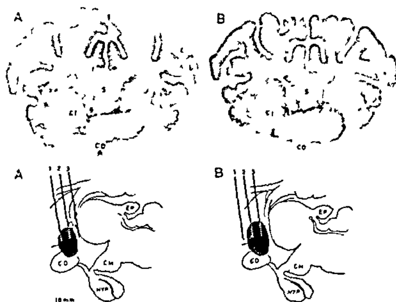


Fig 1 *The final extent of the preoptic lesions in the proton irradiated animals (goats A and B)*
Below Drawings of mid sagittal sections through the hypothalamus showing the position of permanently implanted electrodes (1, 2 and 3) and the sagittal extent of the lesions (*black*) *Above* Photographs of transverse sections through the brains of the two goats at the level of and in the direction of electrodes no 2 Stain Toluidine blue

CI = Capsula interna	Ep = Epiphysis
CM = Corpus mammillare	Hyp = Hypophysis
CO = Chiasma opticum	S = Septum pellucidum

bilateral lesions in this part of the brain often have lethal effects, causing fatal hyperthermia and hemorrhagic edema of the lungs (Gamble and Patton 1953) By vigorous therapeutic and nursing care Keller (cf Keller 1963) has succeeded in getting dogs to survive after extensive preoptic/anterior hypothalamic lesions Careful long term studies of temperature regulation in these animals have provided new information on the importance of the rostral hypothalamus in the regulation of body temperature Keller has found that dogs with preoptic lesions exhibit a considerable impairment in the regulation against heat Further the dogs with preoptic/anterior hypothalamic lesions also show an impaired regulation against cold and become adipsic

Concomitant with studies of the thermoregulatory responses to local cooling and warming of the preoptic anterior hypothalamic region in the goat (cf Andersson Gale and Hokfelt 1964 b acute and chronic effects of lesions of this part of the brain have been studied over the past 5 years To a great extent the observations made are in agreement with Keller s observations in the dog The two techniques used to produce local brain lesions in the goat however have made it possible to follow without the interference of anesthesia 1 the effects of gradually developing preoptic proton lesions or (2) the immediate effects of rapidly induced coagulative lesions The experiments reported here therefore are in some respects complementary to Keller s studies in the dog



Fig 2 X ray pictures of the skull of goat VI showing the four thermo-couple electrodes implanted with their uninsulated ends in the preoptic region (the rostral pair) and in the mid hypothalamic region (the caudal pair)
Left Side view *Right* Dorsa
The pictu
used for RF
regions
CO The position of the optic chiasma
CM The position of the mammillary body

Methods

blood temperature

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two perpendicular

axes, care was taken to distribute the radiation dose so as to produce eventually an extensive [illegible]

physical conditions as applied for destruction of the median eminence in the work by Gale and Larsson (1963), i e a = 3 mm, b = 6 mm, λ max = 40°, β max = 20° (for definition of the parameters see Leksell *et al* 1960)

After completion of the irradiation the goats were moved back to Stockholm and placed in their customary environment Thereafter the respiratory response to electrical stimulation of the "heat loss center" and the electrical activity in this part of the brain were recorded daily for a period of 25 days By following the gradual disappearance of the panting response to stimulation, and by determining the vanishing point of neuronal activity recorded between the two effective heat dissipation electrodes", it was possible to obtain a certain idea of the onset of and the spread of tissue destruction in the irradiated part of the brain

Injections of pyrogen The response to exogenous pyrogen was tested by administration of pseudomonas polysacharide (Piromen, Travenol Lab) intravenously in a dose of 1 1 μg/kg b w [illegible]

B Animals with radio-frequency (RF) lesions

Six adult female goats (in the following referred to as I to IV) were used Goats I and IV had a pair of circular silver plates implanted bilaterally in the internal capsula so that the cylinder of brain tissue limited by the two plates included the preoptic region A needle applicator for temperature recording was also implanted with its thermosensitive tip in the center of the cylinder bounded by the silver plates Goats II, III, V and VI had two pairs of thermocouple electrodes implanted bilaterally in the preoptic/anterior hypothalamic region (Fig 2) The [illegible] and urinary output were made

Production of lesions Coagulative brain lesions were made by applying RF energy between the silver plates or between the uninsulated ends of the thermocouple electrodes (two at a time and in all possible combinations) The temperature in the heated part of the brain was raised to a level of 65 to 70° C The procedure took about 5 min in the goats supplied with silver plates and about 15 min in the others The coagulation was performed without anesthesia and with the goats standing in the metabolism cages It was not seen to cause pain or obvious distress to the animals The only effects observed during the actual heating of the preoptic region were strong panting and a strong urge to drink water

Chemical methods Plasma sodium was determined by use of an EEL flame photometer and plasma chloride was determined according to Brun (1949) Blood glucose was determined enzymatically as described by Levin and Linde (1962) Urine was collected when normally voided and urinary contents of adrenaline and noradrenaline were estimated fluorimetrically according to the procedure described by Euler and Lishajko (1961) Radioiodine (I^{131}) was used to study thyroid activation by methods previously reported (cf Andersson *et al* 1963a)

Histology The same histological technique was used as in the animals with proton lesions

Results

A Animals with proton lesions

The post-irradiation studies were continued for 83 days in goat A and for 54 days in goat B, after which time the animals were killed At the time of sacrifice, goat A was in good condition whereas goat B was in a poor nutritional state due to senso-motor disturbances affecting its food intake (see below)

1 Indications of the onset and the spread of preoptic tissue destruction

The development of tissue destruction in the preoptic region was indicated by a gradual disappearance of the panting and vasodilatation response to electrical stimulation of the "heat loss center". In goat A this response to stimulation was reduced on the 7th post irradiation day and had vanished completely on the 12th day after proton irradiation of the preoptic region. The corresponding times in goat B were the 6th and the 10th post irradiation days. Concomitant with the diminishing response to electrical stimulation of the "heat loss center" there was a gradual reduction of the electrical activity recorded between the electrodes 1 and 2 in goat A and electrodes 2 and 3 in goat B (for electrode placement see Fig. 1). After the 12th post irradiation day activity was no longer recorded at this site in goat B and hardly any in goat A. The lateral spread of tissue destruction to parts of the internal capsula became dramatically evident in goat A on the 11th post irradiation day. At 8 a.m. that day the goat started to lift its right foreleg and turn its head towards the right. Maximal right turning of the head and circling to the right when kept free in a pen were seen 2 hours later. At 11 a.m. the goat again held its head in normal position and no longer circled to the right. From noon until 3 p.m. the identical motor disturbances appeared on the contralateral side. From then on no obvious motoric disturbances were observed in goat A during the remaining 72 days of observation.

The occurrence of motor effects in goat B was less dramatic. Lifting of the right foreleg was observed on the 5th post irradiation day and a similar contralateral effect on the 7th day after irradiation. From then on the goat exhibited muscular weakness and some difficulties of standing and impaired ability to grasp hay with the lips and to chew it. These motor disturbances remained until the goat was sacrificed on the 54th post irradiation day and may explain the reduced food intake observed in this goat especially during the later part of the post irradiation period.

It was thus obvious that the brain lesion in both animals had spread to involve the tips of the two "heat dissipation electrodes" and laterally so far as to involve parts of the internal capsula on both sides within 14 days after proton irradiation of the preoptic region. A further spread to involve electrode 1 in goat B and electrode 3 in goat A did not occur. This was shown by the histological examination of the brains (Fig. 1) but was also evidenced by the effects of unipolar electrical stimulation via these two electrodes. The tip of electrode 3 in goat A was situated in the mid hypothalamic region just lateral to the caput of the right paraventricular nucleus. Stimulation via this electrode (0.2 mA, 50 c/s) caused slight licking during the pre irradiation period. From the 12th post irradiation day this response was changed into shivering and the shivering response to stimulation remained until the goat was sacrificed. In goat B the response to electrical stimulation via electrode 1 (weak chewing movements) remained unchanged until the goat was sacrificed.

2 Alimentary effects

The alimentary effects of the preoptic proton lesions consisted of complete adipsia from about the 8th post irradiation day and onwards and of a lack of thermal inhibition of food intake. These effects have been described and discussed earlier (Andersson and Larsson 1961, Andersson, Gale and Sundsten 1964 c). In addition goat B exhibited the senso-motor disturbances of eating described above.

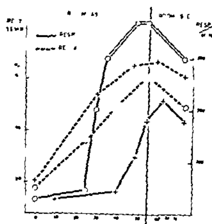


Fig 3 A comparison between the responses to heat exposure (45° C) before (*circles*) and after (*crosses*) destruction of the preoptic region by proton irradiation in goat B Note that open mouth panting (*double lining on respiration curve*) [illegible] when rectal temperature had reached 41 C.

3 Thermoregulatory effects

For the first 25 post irradiation days the goats were kept in the same rooom as during the pre-irradiation period During the initial 14 days the temperature of the room remained at 18 ± 1 C In this external environment the rectal temperature within three days after irradiation rose from a normal level of about 39 0° C to a level fluctuating between 40 0 and 40 5° C and remained hyperthermic until the 14th post irradiation day At this time the onset of autumn weather caused great fluctuations of the temperature of the animal room, which at night reached as low as 13° C It was then observed that the rectal temperatures of both goats become hypothermic and erratic tending to follow the fluctuations in room temperature For this reason both animals on the 26th post irradiation day were moved into a room in which the temperature was maintained constantly at 25 C Both goats remained in this external temperature for the remainder of the observation period with the exception of brief periods of time when their responses to heat and cold exposure and to pyrogen were tested At 25° C the rectal temperature of the goats remained about 1 C above normal level

Responses to heat load The response to an external heat load heat chamber at 45° C) was tested on the 21st and 82nd post irradiation day in goat A and on the 24th post irradiation day in goat B Both goats had in similar tests performed during the pre-irradiation period started to pant with open mouth (resp freq 300—370 min) when the rectal temperature reached a level of 40 3 to 40 7 C During post irradiation heat exposure no open mouth panting occurred in spite of rises in rectal temperature to between 41 2 and 41 5 C However an increase in respiratory frequency to about 200 min was seen during the post irradiation heat tests when rectal temperature reached a level of about 41 C Fig 3 It was thus evident that a certain ability to respond to hyperthermia with polypnea remained in the goats after destruction of the preoptic region

Responses to cold After it had been observed that the irradiated animals also showed an impaired regulation against cold their responses to brief periods of cold exposure were repeatedly tested For comparison parallel studies of the response of normal goats were made on each occasion The tests were made either at 5 C or at − 10 C When exposed to cold the normal animals soon started to shiver and thus maintained

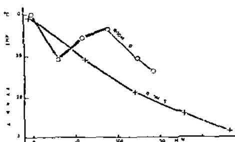


Fig 4

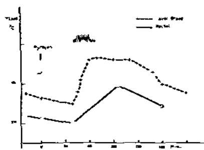


Fig 5

Fig 4 A comparison between the effects of moderate (*crosses*) and severe (*circles*) cold exposure on deep body temperature in goat B after destruction of the preoptic region Shivering is indi-

better maintained

The blood temperature in the anterior caval vein was measured about 5 cm from the heart.

Fig 5 *Fever response to pyrogen after complete destruction of the preoptic region*

The experiment was performed on the 80th post irradiation day in goat A Pseudomonas polysaccharide (Piromen, Travenol Lab) was given intravenously in a dose of 1 1 g/kg b w (at the arrow) The temperature of the blood was measured in the anterior caval vein about 5 cm from the heart

or even raised their rectal and caval vein temperatures slightly In the proton irradiated animals when exposed to moderate cold (+ 5° C) there was a continuous drop in these temperatures to a level of between 36 and 37° C Only at this degree of hypothermia did moderate shivering appear, preventing a further drop and sometimes causing a rise in body temperature Such shivering dramatically grew very strong when the goats were led back into the warm room apparently facilitated by the forced muscular exercise

Surprisingly enough the irradiated goats were better able to maintain body temperature during relatively short (2 hours) exposure to severe cold (— 10° C) At this temperature shivering became apparent much sooner and grew rather strong A comparison between the effects of moderate and severe cold exposure on caval blood temperature in goat B is given in Fig 4

Responses to pyrogen The responses of the proton irradiated goats to exogenous pyrogen were tested on the 42nd post irradiation day in goat B and on the 80th post irradiation day in goat A, *i e* on the 12th and 3rd day before the goats were sacrificed At this time the lesions of the preoptic region were likely to have reached the extent found in

vein blood temperature had fallen to normal levels at the time of injection of pyrogen The intravenous injection of pyrogen was in both irradiated goats found to cause the similar febrile response as in intact goats After a latency time of about 40 min the animals started to shiver and the rectal and caval vein blood temperatures rose by about 1° C and remained elevated for about two hours (Fig 5)

TABLE I

Site of lesion	Goat no	Maximal post lesioning rectal temp °C	Initial shivering and periph vasoconstr	% Change in blood glucose
Preoptic region	I	42 1	+++	+ 300 at 7 hours
Preoptic region	II	42 5	+++	+ 400 at 10 hours
Preoptic region	III	42 5	+++	+ 100 at 6 hours
Preoptic region and anterior hypothalamus	IV	41 4	+++	+ 400 at 10 hours
Preoptic region and anterior hypothalamus	V	41 9	+++	+ 60 at 30 hours
Preoptic anterior hypothalamic and mid hypothalamic regions	VI	Hypothermia 36 4 at 10 hours	None	— 70 at 6 hours

4 Histology

The histological examination of the brains of the proton irradiated goats revealed extensive bilateral lesions involving the entire preoptic region The extent of the lesions are shown on microphotographs of transverse sections through the preoptic region and on drawings of midline sagittal sections in Fig 1 The lesions in both goats were slightly larger on the left side The destroyed region was larger in goat B than in goat A In goat B it involved more of the internal capsula which may explain why senso-motor disturbances appeared in this animal In neither goat did the lesion extend so far caudally into the anterior hypothalamus as to touch the descending column of the fornix or the paraventricular nuclei

B Animals with RF lesions

Destruction of the preoptic and the preoptic hypothalamic region by RF heating in the unanesthetized goat produced three different types of derangements in thermal homeostosis which could be correlated with the degree to which the lesions extended

% Change in urinary noradrenaline (NA) and adrenaline (A) excretion	% Change in plasma PBI[131]	Comments
Not studied	+ 8 at 2 hours	Dead at 11 hours Pulmonary edema
NA +150 A +850 at 12 hours	+ 100 at 10 hours	Dead at 12 hours Symptoms of pulmonary edema
NA +85 A 1 000 at 12 hours	+ 100 at 6 hours	Dead at 16 hours Symptoms of pulmonary edema
Not studied	− 25 at 7 hours	Hyperthermic and hyperactive for 48 hours. Then normothermic and sluggish Killed on day 5 in poor condition
Day 3 NA +165 A ± 0 Day 4 NA +254 A +100 Day 20 NA ± 0 A + 0	Not studied	Initial hyperthermia for 7 days Then impaired regulation against both heat and cold Adipsia Killed in good condition after 8 months
NA −100 A 20 at 10 hours	Not studied	Hypothermic Somnolent. Adipsic Killed on day 6

caudally into the hypothalamus The effect of the lesions in the six animals studied are summarized in Table I

I Lesions mainly restricted to the preoptic region

In goats I II and III the coagulative lesions were found at histology to be confined primarily to the preoptic region. The extent of the lesion in goat III is shown in Fig 6 In marked contrast to the slow onset of moderate hyperthermia induced by progressive inactivation of the heat loss center' by proton irradiation the abrupt coagulation of the preoptic region by RF heating in these three goats resulted in an immediate and marked mobilization of neuromuscular and hormonal thermogenic mechanisms Thus upon completion of the RF heating the animals typically exhibited strong shivering and intense vasoconstriction even though room temperature was above 20° C. The intensity of shivering gradually diminished as the rectal temperature rose, and shivering ceased within 3 hours by which time rectal temperature had reached 41° C. At this level of rectal temperature moderate polypnea appeared The rectal tempera

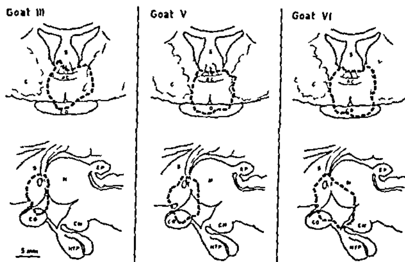


Fig 6 *Examples of the three types of preoptic/hypothalamic RF lesions studied*
Above Drawings of transverse sections through the preoptic region at the level of the anterior commissure (A C)

text

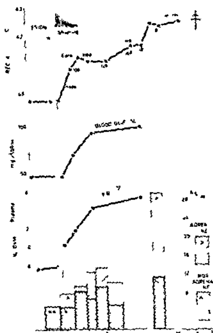
AC = Anterior commissure
CI – *Capsula interna*
CM = Corpus mammillare
CO = Chiasma opticum

Ep = Epiphysis
Hyp = *Hypophysis*
MI = Massa intermedia
S = Septum pellucidum

ture continued to rise to 42 5 C in association with persistent peripheral vasoconstriction (indicated by low ear surface temperature) and increase in respiratory frequency to 150—200 per min

During the initial period of most rapid temperature rise the goats remained alert and occasionally ate hay and ruminated After 3 to 6 hrs they became increasingly restless and agitated showing marked forward progression It was necessary to restrain them in order to prevent their pitching forward out of the metabolism cages

Associated with the rapid extreme post lesioning activation of neuromuscular heat production there occurred also a marked activation of hormonal thermogenic mechanisms Within 3 hrs plasma protein bound I^{131} (PBI^{131}) and blood glucose levels were strikingly elevated and continued to rise as hyperthermia progressed (see Table I) The two goats (II and III) in which urinary excretion of catecholamines were followed showed a progressive increase in sympathico-adrenomedullary activity with a particularly *striking elevation of adrenaline the normal ratio of noradrenaline* to adrenaline being reversed Terminally adrenaline excretion had increased about 10 times whereas noradrenaline excretion was doubled



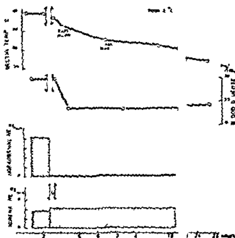


Fig 7 Fig 8

Fig 7 *Effects of acute RF destruction of the preoptic region in goat III*

The extent of the lesion is shown in F g 6
of the preop
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Fig 8 *The acute effects of RF-destruction of the preoptic the anterior hypothalamic and the mid hypothalamic regions in goat VI*

The extent of the lesion is shown in Fig 6

Note that the extension of the preoptic lesion caudally to involve also the mid hypothalamic region converted sympathetic release into an inhibition (virtual disappearance of urinary excretion of *noradrenaline* hypoglycemia and hypothermia)

Death occurred in these goats at 11 12 and 16 hrs post lesioning and seemed to be due to pulmonary edema and hyperthermia Goat I showed a profuse mucoid salivation and rales terminally The effects of destruction of the preoptic region in goat III are shown in Fig 7

2 Lesions involving the preoptic and the anterior hypothalamic regions

Two goats IV and V in which the RF lesions besides destroying the preoptic region, extended caudally to involve the anterior hypothalamus survived the acute hyperthermia after induction of the lesions The extent of the lesions in goat V is shown in Fig 6 The initial response of these two goats to brain coagulation resembled that

described above — shivering and peripheral vasoconstriction and development of considerable hyperthermia associated with moderate polypnea Rectal temperatures however, did not rise quite so high as in animals with RF-lesions restricted to the preoptic region, nor did the animals seem to become so agitated

Goat IV remained hyperthermic and hyperactive for 48 hrs It then became normothermic and sluggish and developed gastro-intestinal stasis It was killed in poor condition 5 days after induction of the lesion

In goat V the acute hyperthermia persisted for 7 days after lesioning On the 2nd and 3rd days the urinary excretion of noradrenaline rose to peaks of 165 % and 254 % above control while adrenaline increased 100 % At this time blood glucose was elevated 40 % During this period of hyperthermia (rect temp 40 7 to 41 2° C), respiratory rate was maintained at 60—96/min, *i e* moderate polypnea The gradual subsidence of hyperthermia occurred in association with a decline in urinary excretion of catecholamines Thus, on day 8 post lesioning, when rectal temperature had fallen to 40 3° C, catecholamines in urine were only slightly elevated, and by day 30 were at pre-lesion levels At this point the animal showed impaired regulation against both heat and cold and continued to do so until sacrificed 8 months post lesioning When *maintained in its customary environment (at 21 ± 1° C) its rectal temperature* stayed about 1° C above normal, when placed at 18° C the goat became normothermic, and when exposed to cold the rectal temperature fell to hypothermic levels As in the animals with proton lesions, the shivering response to moderate cold exposure was absent or very weak in spite of a fall of rectal temperature below 36° C, whereas the shivering response to severe cold exposure was more accentuated

The alimentary effects in goat V were also similar to those of the goats with proton lesions The goat was thus permanently adipsic Its necessary water intake was maintained by providing a grain-water mixture daily, which the animals drank voluntarily In order to test the completeness of adipsia, this fluid on two occasions was withheld for a 3 day period while drinking water was continuously available During these periods of dehydration the goat never drank water even though plasma concentrations of Na and Cl rose to 196 and 160 meq/l, respectively

3 Lesion extended to involve the mid hypothalamic region

A large RF lesion involving the preoptic, the anterior hypothalamic, and the mid-hypothalamic regions was induced in goat VI The extent of the lesion is shown in Fig 6 In marked contrast to the RF lesions restricted to the more anterior part of the brain this lesion immediately resulted in hypothermia The rectal temperature fell 2 6° C within the first 2 hrs after lesioning in association with the development of somnolent behaviour, peripheral vasodilatation and a fall in blood glucose to 20 mg/100 ml from a normal level of about 50 mg/100 ml By 12 hrs post-lesioning rectal temperature was 36 5 C Sympathico-adrenomedullary activity was reduced during this interval as indicated by a virtual disappearance of noradrenaline from the urine Adrenaline values were practically unchanged The acute effects of the lesion are shown in Fig 8

Although maintained at 21 ± 1 C goat VI remained hypothermic (34 C rectal temperature) during the next 6 days Within this period of time the blood glucose level gradually returned towards normal I v injection of pyrogen (1 1µg/kg b w of Piromen) on day 5 post lesioning resulted in a 0 5° C rise in rectal temperature within 1 hr but without inducing shivering Because of showing extreme sluggishness the goat was killed 7 days after induction of the lesion

Discussion

In a long series of ablation and transection experiments in the dog Keller and coworkers (cf Keller 1963) have provided invaluable information on the spatial organization of central control of body temperature The results may be summarized as follows *Complete poikilothermia* is obtained by wide symmetrical lesions destroying the junctional tissues between the hypothalamic grey and the habenular tracts, or by complete transection of the brain stem at any level caudal to the cephalic portion of the midbrain *Poikilothermia against heat* with near normal regulation against cold is seen as result of a transection of the brain stem at the level of the pons leaving only the pyramidal bundles intact The opposite, *poikilothermia against cold* with retention of normal regulation against heat is obtained by tissue defects destroying all the posterior hypothalamic grey, or by a 3/4 ventral transection of the brain stem at the pontine level Transections of the brain in front of the preoptic region, on the other hand, do not much interfere with the regulation of body temperature It is thus evident that the hypothalamic heat dissipation and heat conservation outflows may be separated at the midbrain pontine level

Of particular interest for the discussion of the present results in the goat are Keller's observations on the effects of lesions restricted to the preoptic/anterior hypothalamic region in the dog In contrast to what might be expected from earlier studies of the effects of preoptic lesions (Teague and Ranson 1936, Clark, Magoun and Ranson 1939) Keller found that such lesions do not cause a lasting complete poikilothermia against heat although the result may be a permanently raised heat dissipation threshold The same holds true for large lesions involving most of the hypothalamus Another interesting observation of Keller's is that dogs surviving lesions in the preoptic region may also develop an impaired regulation against cold and may become adipsic.

It is, however, extremely difficult to obtain survival after large bilateral lesions restricted to the preoptic region It may be argued, therefore, that if an animal with an appropriate preoptic lesion could be kept alive, it would become completely and permanently poikilothermic against heat The results of the proton lesions in the goat indicate that such is not the case Proton irradiation was selected as the experimental tool for two main reasons It was thought that the gradual development of brain destruction would give the animals time to "compensate" for otherwise lethal effects of a complete destruction of the preoptic region Secondly the absence of anesthesia would avoid any masking of the effects of the lesion By the use of proton irradiation it was in fact, possible to obtain goats surviving brain lesions of a size and a localization (Fig 1) that would have been lethal if induced acutely in a one stage operation The end result of the proton irradiation in both goats was a complete destruction of the tissue which on electrical stimulation responded with the activation of heat dissipation mechanisms (the "heat loss center") Still, these goats responded to hyperthermia with

[illegible]

with acute RF lesions restricted mainly to the preoptic region stopped shivering and responded with polypnea when the body temperature reached about 41° C

After an initial period of constant hyperthermia at neutral room temperature it became evident that the destruction of the preoptic region in the irradiated goats had also caused impaired regulation against cold Local cooling of this part of the brain

[illegible]

tion of "warmth detectors" and stimulation of "hypothermia detectors" (Hammel *et al* 1960) The eventual impaired ability of the animals to maintain body temperature in a cold environment after preoptic lesions speaks in favour of the latter concept The preoptic region may therefore be regarded not simply as a "heat loss center" but rather as a "thermoregulatory center" of importance both in heat and cold defence Recent microelectrode studies have in fact provided direct evidence for the presence in this part of the brain of two kinds of thermosensitive neurons (1) those reacting specifically to a rise in local temperature, and (2) neurons reacting specifically to a fall in local temperature The latter are numerically much fewer than the former (Hardy, Hellon and Sutherland 1964)

Although reduced, cold defence was by no means abolished in the goats with proton lesions Thus only moderate hypothermia developed when the animals were exposed to cold. It was also observed that forced movement of the goats strikingly accentuated their shivering response during hypothermia Keller (1963) observed the same phenomenon in mid thalamic preparations and it is evident that his conclusions also hold true for the goat, namely, that "there is a central segment of the heat conservation outflow which is temperature dependent but is not thermally activated" As in normal animals shivering in the preoptically lesioned goats was also driven by the inflow from peripheral cold receptors since exposure to severe cold induced shivering at a much milder degree of core hypothermia than did exposure to moderate cold (Fig 4)

A widely held view on the central action of pyrogens is that their febrile effect is due to a direct inhibitory action on the preoptic "heat loss center" This would mean a resetting of the regulated temperature to a higher level The observation of Andersen Hammel and Hardy (1961) that the hyperthermic effect of preoptic cooling is potentiated by pyrogen has been taken as a support for this view However the two goats with proton lesions showed a marked febrile response to pyrogen, indicating as do the experiments of Chambers *et al* (1949), that pyrogens may act at a lower level of the brain to cause fever

The alimentary effects in the goats surviving preoptic destruction were adipsia and a lack of thermal inhibition of food intake This is in agreement with the alimentary effects of experimentally induced local temperature changes in the preoptic/anterior hypothalamic region and indicates that there exists an interrelation between the regulations of body temperature and of food and water intake (cf Andersson 1963)

The use of permanently implanted electrodes made it possible to study the immediate effects of preoptic and preoptic hypothalamic lesions in unanesthetized goats The explosive nature of fatal hyperthermia developing after RF lesions restricted to the preoptic region illustrates the powerful inhibitory influence normally maintained by this part of the brain on neuromuscular and hormonal thermogenesis. The occurrence of immediate strong shivering and peripheral vasoconstriction and the quick mobilization of hormonal thermogenesis are consistent with an abrupt release from preoptic inhibition of hypothalamic "centers" subserving thermogenesis That this is due at least in part to a destructive inhibition of "warmth detectors" is indicated by the fact that preoptic cooling gives a similar although less pronounced response (cf Andersson *et al* 1964 b During preoptic cooling also the relative increase in urinary excretion of adrenaline is greater than that of noradrenaline Andersson *et al* 1963 b The massive

sympathico-adrenomedullary activation immediately following upon preoptic RF-destruction was probably the main cause of the fatal hyperthermia and pulmonary edema seen in goats I, II and III This would be in agreement with the observation that the development of pulmonary edema after preoptic lesions in the rat may be prevented by cutting of the splanchnic nerves or by cervical spinal transection (Maire and Patton 1956 a and b) Ganglionic blocking agents prevent the development of hyperthermia during preoptic cooling in the goat (Andersson *et al* 1964 a) and it would be of interest to study whether they would also prevent or delay the lethal effects of acutely induced preoptic lesions.

The caudal extension of the preoptic RF-lesions to involve the anterior hypothalamus apparently reduced the intensity of the initial sympathico-adrenomedullary response, which may explain why such lesions caused less pronounced hyperthermia and permitted survival of the goats It would appear that the partial destruction of hypothalamic sympathetic "centers" resulted in a dampening of the release phenomenon Similarly, the extension of the lesion further caudally to involve also the mid hypothalamic region (goat VI) converted sympathetic release into an inhibition and caused and immediate fall in body temperature.

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Periodic Variation in the Amount of Semen Yielded at Spontaneous Ejaculations of the Male Rat

By

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Abstract

J E Kihlstrom *Periodic variation in the amount of semen yielded at spontaneous ejaculations of the male rat* Acta physiol scand 1965 65 61—64 — Male rats have been prevented from grooming the penis by means of a stiff girdle and the spontaneously ejaculated semen has been collected The amount of semen yielded varies rhythmically with a mean period of 4 4 days

Rhythmical variations in male sexual functions have been demonstrated in men bulls and rabbits (Doggett 1956 Doggett and Keilers 1962 Kihlstrom 1958 1962 1963, Degerman and Kihlstrom 1961 1964 Kihlstrom and Hornstein 1964 a b Kihlstrom and Hultnas 1965) In the rabbit at least some of these variations are dependent upon testicular hormones (Kihlstrom and Degerman 1962 Hornstein Kihlstrom and Degerman 1964 Kihlström and Hornstein 1964b) In all these studies semen was collected with constant intervals of time by means of an artificial vagina This regular handling introduces an external rhythm which may induce or influence the cyclic phenomena studied Rats however ejaculate spontaneously without external stimulation and the yielded ejaculates appear as plugs of coagulated semen which may be collected Orbach 1961) Consequently it will be possible to obtain information whether or not cyclic variations occur also in spontaneous sexual activities

Material and methods

diurnal rhythm with a dark period extending from 8 p m to 8 a m Below the net floor of each cage was spread a paper from which the seminal plugs were collected Each cage was connected with a timer and equipped with an electromagnet automatically placing a sheet of paper below the floor at 8 p m 2 a m etc Thus the ejaculates could be collected in batches

TABLE I The found distribution of the lengths of the gaps between maxima compared with the theoretical distribution to be found in a series of random numbers

Length of the gaps in days	Number of gaps found (=F)		Number of gaps to be found in the theoretical distribution (=T)		$\frac{(F-T)^2}{T}$
0 50	33		57 20		10 24
0 75	32		47 66		5 15
1 00	37		24 51		6 36
1 25	15	41	9 54	13 64	54 98
1 50	6		3 03		
1 75	6		0 82		
2 00	7		0 19		
2 25	3		0 03		
2 50—3 25	4		0 03		
				χ^2	76 73
					$p < 0\ 0005$

two from each light and dark periods The papers were removed once a day and the plugs dried over silica gel to constant weight As the plugs vary considerable in size, the total weight of the plugs yielded per period was taken as the most suitable measure of the activity of the sexual organs

The statistical method used in discovering cycles has been described earlier (Kihlstrom 1962, 1963 Degerman and Kihlstrom 1964) and will only be shortly reviewed The collected data were arranged in chronological order with a constant interval of the time six hours In the series all maximum and all minimum values as to the total weights of the plugs were marked The distribution of the lengths of the gaps between these maxima has been compared with that found in a series of random numbers (Kermach and McKendrick 1937) More detailed information was obtained by rearranging the series in horizontal rows of identical length one below the other This arrangement was repeated, the number of figures in each row varying from 3 to 25 Unless the collected data express cyclic variation, the maximum values will be distributed at random among the vertical columns obtained by the procedure described A cyclic variation of a given length is however manifested as an accumulation of the regularly recurring maximum values in one column This happens when the horizontal row has a length approaching that of the cycle or of a multiple of the cycle The probability that a given frequency of maxima in a vertical column is statistically different from that expected if the values were distributed at random among the columns has been tested as described earlier (Kihlstrom 1962 1963) In this test small maxima are given the same importance as those deviating more markedly from their adjacent values The mean weight calculated from the column with the regularly recurring maxima was therefore compared with that calculated from the column having the regularly recurring minima by means of t test The shortest row giving significant cyclicity and a significant difference in mean weights between columns with recurring maxima and minima was regarded as approximating the length of the cycle

Results

The experiments lasted from April 21 to June 8 In most cases at least one ejaculate per day was yielded Plugs were found during 18 per cent of the six hours periods the highest number per period being 6 plugs and the mean value 1 2 plugs In all animals

TABLE II Statistical analysis of the cyclic phenomena in the weights of spontaneously yielded ejaculates of male rats

Rat no	Test for regularly recurring minima: Period length in days	t	f	p <	Difference between columns with maxima and minima, respectively: t	f	p <
I	3.25	2.10	141	0.025	1.84	20	0.05
II	4.25	2.14	84	0.075	2.18	9	0.05
III	5.50	1.95	149	0.05	2.07	11	0.05
IV	4.00	3.17	109	0.005	3.58	12	0.005
V	4.50	1.73	150	0.05	1.90	16	0.05
	5.50	1.73	150	0.05	2.06	12	0.05
VI	3.25	2.57	167	0.01	1.81	18	0.05
	3.50	2.09	166	0.025	1.79	18	0.05
	4.50	2.09	166	0.025	3.70	14	0.005
VII	2.50	1.75	164	0.05	2.12	28	0.025
	4.50	1.83	157	0.05	1.91	14	0.05
VIII	5.25	1.73	134	0.05	2.60	10	0.075

the greatest number of plugs was delivered between 8 p.m. and 2 a.m. (Kihlström 1965).

As seen from Table I the distribution of the lengths of the gaps between peaks in the weights of the plugs is far from haphazard which indicates the occurrence of cyclic phenomena. Table II gives the results of the statistical analysis using the method for testing regularly recurring maxima. All animals show statistically significant cycles having significant differences between columns with recurring maxima and minima, respectively. In an earlier paper (Kihlström 1965) a diurnal rhythm in the spontaneous ejaculations of the rat has been demonstrated. This distinct rhythm with a length of 4 intervals of course influences the longer cycles and most cycles with a length being a multiple of 4 are statistically significant. In order to avoid this influence by the diurnal rhythm all these cycles have been excluded. This of course decreases the probabilities found. However in one animal, number IV, only these cycles are significant, the most pronounced one having a length of 4.0 days (16 intervals). This figure perhaps represents the length of the cycle in this individual. Three animals, number V, VI and VII, exhibit 2 or 3 significant cycles of somewhat different lengths. This may depend upon varying lengths of individual cycles. The lengths of the most pronounced cycles range from 3.25 to 5.50 days with a mean of 4.4 days.

Consequently the spontaneous activity of the sexual organs of the male rat varies rhythmically. As this periodicity occurs in the spontaneous delivery of ejaculates during constant conditions the regulating mechanism ought to be endogenous, probably of hormonal nature.

To the Head of the Institute, Professor P E Lindahl my thanks are due for valuable advice and discussion For construction and building of the cages I am obliged to Mr E Nyberg Dr J C Månsson has been helpful in plastering the animals Financial support from the Swedish Natural Science Research Council is gratefully acknowledged For technical assistance I thank Miss Ingvor Andersson

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From the Nobel Institute for Neurophysiology, Karolinska Institutet Stockholm 60 Sweden

The Adaptation and the Relation between Discharge Frequency and Current Strength of Cat Lumbosacral Motoneurones Stimulated by Long-Lasting Injected Currents

By

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Abstract

Kernell D *The adaptation and the relation between discharge frequency and current strength of cat lumbosacral motoneurones stimulated by long-lasting injected currents* Acta physiol. scand 1965 *65* 65—73 — Cat lumbosacral motoneurones were stimulated by steady currents injected through the tip of an intracellular micro-electrode The results strongly indicate that the majority of undamaged motoneurones are capable of responding to steady currents by long lasting and well maintained repetitive discharges Such discharges from various motoneurones were stud ed with regard to the adaptation and the slope of the linear relation between discharge [illegible]

muscle stretch

Granit Kernell and Shortess (1963 *a b cf* also Shapovalov 1964) studied the repetitive discharges set up in motoneurones by long lasting injected currents They were mainly concerned with cells which discharged repetitively in a well maintained manner (continuously discharging cells) The stimulus threshold for evoking continuous firing of a neurone was always higher than the rheobase and the relation between discharge frequency and current strength (the f I relation') was found to be linear over a considerable range The firing rate as well as the value of the proportionality constant for the linear f I relation (the f I slope) decreased by variable amounts during the initial part of continuous neurone discharges (the initial phase of adaptation) Sometimes the firing rate would also decrease during later periods of the discharge but during this late

phase of adaptation", the *f I* slope stayed constant (Granit *et al* 1963 *b*) However, many of these findings concerning continuous neurone discharges could not be directly applied to the repetitive firing of cat motoneurones as most of the results of Granit *et al* (1963 *a*, *b*) were obtained from the rat, and because an unknown proportion of their motoneurones would discharge only phasic burst in response to long lasting currents ("transiently discharging cells")

One aim of the present work was to extend the quantitative analysis of repetitive firing (Granit *et al* 1963 *a*, *b*) to a greater number of cat motoneurones Another aim was to investigate characteristic differences in repetitive firing properties, such as those summarized above, and to correlate them with the duration of afterhyperpolarization as measured by antidromic spike discharges This latter part of the investigation was inspired by the earlier findings that motoneurones, which respond to muscle stretch by maintained repetitive firing (the "tonic" units of Granit, Henatsch and Steg 1956 Granit, Phillips, Skoglund and Steg 1957) have long afterhyperpolarizations (Eccles Eccles and Lundberg 1958, Kuno 1959, Eccles, Eccles, Iggo and Ito 1961) Previous work has also strongly indicated that the repetitive firing elicited by maintained reflex activation is set up chiefly by comparatively steady synaptic currents (e g Eccles 1953, p 174 ff, 1964 p 114 ff, Frank and Fuortes 1961, Granit and Renkin 1961) Due to differences in the synaptic organization (cf Eccles, Eccles and Lundberg 1957), the same muscle stretch would perhaps produce synaptic currents of greater strength and duration in the "tonic" than in the "phasic" cells A possibility also to be considered however, is that muscle stretch produces similar currents in both types of cells but that the "phasic" neurones (which have short afterhyperpolarizations), because of inherent membrane properties, are less capable of setting up well maintained firing in response to a steady depolarizing current (*cf* Sasaki and Otani 1961, 1962)

The present results strongly indicate that the majority of undamaged cat motoneurones, whatever their duration of afterhyperpolarization, are capable of setting up continuous discharges in response to long lasting injected currents The quantitative data on maintained repetitive firing as presented in this study, were found to be well in accord with those of Granit *et al* (1963 *a*, *b*) Repetitive firing at higher frequencies, and the limits of discharge frequency will be dealt with in two consecutive papers (Kernell 1965 *a* *b*)

Methods

The results described in this and the two consecutive papers (Kernell 1965 *a* *b*) have been obtained from experiments on 40 cats anaesthetized with pentobarbitone (35–40 mg kg i p) The animals were immobilized with gallamine triethiodide (Flaxedil May & Baker) and maintained on artificial respiration Ventral roots L7 and S1 were cut and their distal portions were placed on stimulating electrodes the spinal cord was covered by warm liquid paraffin Stimulating electrodes were placed on the hamstring popliteal and common peroneal nerves.

Intracellular records were obtained from alpha motoneurones in segments L7 of S1 using conventional single barrelled glass microcapillary electrodes filled with 2M potassium citrate Stimulating rectangular pulse currents were passed through the recording microelectrode (*cf* Araki and Otani 1955 Current strength was monitored by recording the voltage drop across a resistor placed in series with the microelectrode A resistive bridge was used for diminishing shock artifacts. The bridge included a variable potential source to avoid draining current from the cell Conventional cathode followers and d c or a c -coupled amplifiers were used The recording equipment included two double beam oscilloscopes. In some experiments continuous recordings of membrane potentials were taken using a d c recording pen writer full deflection 0 2 sec, Inograph G Type ZSG Rodhe & Schwartz

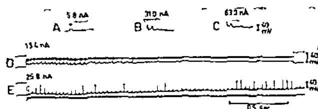


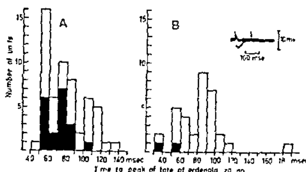
Fig 1 Records of repetitive discharges set up in three different motoneurones by the current strengths indicated A — C from a motoneurone with antidromic spike amplitude 50 mV Time mark 1 000 cps D from a motoneurone with antidromic spike amplitude 50 mV B — C and D show the maximal duration of repetitive discharge in the respective cells E from a motoneurone with antidromic spike amplitude 76 mV The upper beam in D and F signals the strength of current

Results

Transient firing of motoneurones stimulated by long lasting currents

Motoneurones which would respond only by "transient discharges (i e single spikes or brief bursts) at any current strength will be referred to as transiently discharging cells Some transiently discharging cells would only fire one or at most two spikes at any current strength (Fig 1 A C) Other cells responded with brief bursts (Fig 1 D) Such bursts might consist of a few spikes or of a discharge lasting up to about 2 sec Its maximal duration was as a rule obtained at current strengths just above the one needed to elicit repetitive firing On the whole the frequency always progressively decreased throughout the period of discharge but appreciable irregularities in the rate of firing were often observed Throughout the period of discharge there was also a progressive and often quite pronounced diminution of spike size

Motoneurones which at least for some time after penetration would deliver continuous discharges (i e repetitive discharges whose firing rate stays virtually constant



for a long time), will be referred to as continuously discharging cells' (*Fig* 1 E) It should be noted that continuously discharging cells may respond by brief bursts to currents which are either too weak or too strong for eliciting a steady repetitive firing (*cf* Granit *et al* 1963 *a*)

In the present results, 89 lumbosacral motoneurones permitted a careful study of repetitive firing at a number of current strengths At least for some time after penetration, 56 of these cells discharged continuously (Fig 2 A), and 33 cells discharged only single spikes or short bursts (Fig 2 B)

Fig 2 shows that motoneurones responding continuously (A) and transiently (B) to injected currents cannot be clearly distinguished from each other on the basis of differences in the time to peak of late afterdepolarization Time to peak of late afterdepolarization (see Methods) corresponds to the duration of afterhyperpolarization as measured by Eccles *et al* (1958) The average time to peak of late afterdepolarization was 77 ± 22 (S D) msec for the continuously discharging cells in Fig 2 A and 80 ± 26 (S D) msec for the transiently discharging cells in Fig 2 B The difference was not statistically significant (t test $p > 0.5$) Motoneurones with long durations of afterhyperpolarization were comparatively scarce in the present material Many cells with long afterhyperpolarizations were seen, but it was often difficult to obtain stable enough measurements from them (*cf* Kuno 1959) This was probably due to the smaller size of such cells

Granit *et al* (1963 *a*) noted that in some cases a cell early in the experiment responded by continuous firing while later on it only gave a transient discharge or vice versa In the present material also this very often was the case When the resting membrane potential and the spike size decreased just before the final deterioration at the end of a penetration most of the continuously discharging units whatever their duration of afterhyperpolarization delivered only typical transient discharges Such transient discharges were similar to those shown in Fig 1 A D and quite indistinguishable from the transient discharges of the cells collected in Fig 2 B Other cells could initially discharge only transiently but later on became capable of continuous firing This change in the discharge properties was always accompanied by a spontaneous increase of the membrane potential

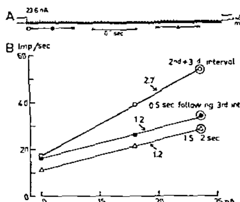


Fig 3 From motoneurone with antidromic spike amplitude 55 mV A Re-

tion Straight lines fitting the experimental values as closely as possible were drawn free hand and the slopes of these lines (imp/sec/nA) are indicated by numbers at arrows

(*cf* Granit *et al* 1963 *a*) The transiently firing units of Fig 2 B were generally unstable and soon deteriorated

These observations indicate that at least many of the transiently discharging cells were depolarized because of damage inflicted by the micro-electrode On this view, large spike sizes should be less common among transiently firing cells. This also proved to be the case Twenty one units out of the present material possessed spike sizes above 80 mV and 19 of these units gave continuous discharges (Fig 2 black areas) The average spike amplitude for all the continuously discharging cells included in Fig 2A was 71 ± ± 14 (S D) mV, for the transiently discharging ones of Fig 2 B, 65 ± 12 (S D) mV The difference between these mean values was significant (t test, 0 05 > p > 0 02) Small spike sizes were also often found in continuously firing units which in all other respects seemed perfectly normal Low absolute values of spike sizes and membrane potentials might however be due either to unknown factors in the recording technique or to excessive depolarization of the impaled unit or to both these factors in combination

It can be concluded from the findings described above that at least the majority of undamaged motoneurones, whatever their duration of afterhyperpolarization (time to peak of late afterdepolarization) would be capable of setting up continuous repetitive discharges in response to injected currents

The rheobase of 18 transiently discharging cells was on the average 9 1 ± 7 0 (S D) nA It was not significantly different from the rheobase for continuously discharging cells (9 2 ± 6 3 (S D) nA, 43 cells) In 12 transiently discharging and 22 continuously discharging cells a notch could be seen on the rising phase of the action potentials in repetitive firing These units showed a distribution to time to peak of late afterdepolarization similar to Fig 2 (*cf* Sasaki and Otani 1961)

f I slopes and adaptation of continuously firing motoneurones

The features of discharges at higher frequencies will be treated in a subsequent paper (Kernell 1965 *a*) For lower discharge frequencies the general findings by Granit *et al* (1963 *a b*) concerning the adaptation and the linearity of the f I relation could be fully confirmed in the present greater number of cat motoneurones

The initial and the late phase of adaptation are illustrated in Fig 3 B The firing frequencies elicited at three different strengths of stimulation were measured at three

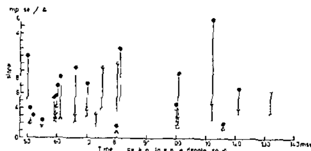


Fig 4 *f I* slopes calculated from the first interval (filled circles) the second interval (triangles) and the steady repetitive firing (circles) of continuous discharges set up by long lasting injected currents *Values from the same* units are interconnected by vertical lines and plotted against time to peak of late afterdepolarization

different times after the onset of current, as marked in the diagram Fig 3 A shows one of the records from which the measurements were obtained In this cell the initial phase of *adaptation lasted only during the first 3—4 intervals*, and the unit thereafter fired according to a f I slope of 1 2 impulses per sec per nA (imp/sec/nA) (Fig 3 B) A further decrease appeared in the firing frequency later on (late phase of adaptation), but the f I slope remained constant The late decrease in rate of discharge thus differs from the early *drop in firing frequency in that it takes place without a change in the value of the* f I slope (*cf* Granit *et al* 1963 *a*, *b*) The f I slope obtained from a continuous discharge after the initial phase of adaptation will be referred to as the 'steady state f I slope" Granit *et al* (1963 *a*) used the term slope constant for the adapted state'

The late phase of adaptation was seen in 15 motoneurones Generally, the discharge frequency decreased slowly during this phase of adaptation Sometimes the late phase of adaptation would be apparent in less than 2 sec after the onset of stimulation (Fig 3), in other cases nothing was seen of it until after 5—12 sec of continuous repetitive activity In most cells the discharge frequency was practically constant for several seconds once the initial phase of adaptation was over

In four motoneurones the repetitive firing was continued for 35 sec or more, and in all these cases the steady state f I slope remained constant throughout (*cf* Granit *et al* 1963 *b*

The initial phase of adaptation of 30 motoneurones lasted for very variable times *It* might be finished during the first 2—3 intervals (0 1 sec or less, cf Fig 3) or it would last up to about one second A quantitative measure of the initial phase of adaptation can also be obtained by comparing the values of the f I slope for the first and second intervals to the values of steady state f I slopes (Fig 4) It is seen in the diagram (*Fig 4*) *that the* relation between these f I slopes was very variable

Fig 4 also shows the range of variation of f I slopes obtained from the first two intervals They were on the average 6 8 ± 3 9 (S D , 17 units) imp/sec/nA for the first interval *and 4 0 ± 2 6 S D 21 units) imp/sec/nA for the second interval*

Steady state f I slopes could be obtained by two different methods Some cells were stimulated by constant currents lasting two seconds or more and often a pause of about half a minute was allowed between each period of stimulation (*cf* Fig 3) Other units were continuously stimulated for several seconds while strength of current was changed step-wise Under steady experimental conditions both methods were compared in 4 motoneurones and were found to give similar values of the steady state f I slope (*cf* Granit *et al* 1963 *b* f I slopes for the first intervals could be obtained only by the former method and only in cases for which the stimulation artifacts were small

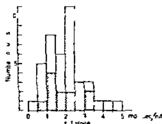


Fig 5 Histogram showing the distribution of steady state *f I* slopes (imp/sec/nA) for the continuous repetitive discharges of 40 motoneurones Hatched areas represent units with time to peak of late afterdepolarization longer than 90 msec

In Fig 5 the distribution of values of steady state f I slopes from 40 motoneurones with continuous discharges are shown The steady state f I slopes varied between about 0 4—4 5 imp/sec/nA and were on the average 1 9 ± 1 0 (S D) imp/sec/nA The present values of steady state f I slopes were on the average somewhat smaller than those found in rat and cat motoneurones by Granit *et al* (1963 *a*)

The variation in adaptation and f I slopes described above, was not related to time to peak of late afterdepolarization i e. to the duration of afterhyperpolarization as measured by Eccles *et al* (1958) For the initial phase of adaptation and for the value of steady state f I slopes this lack of correlation is demonstrated by Figs 4—5 In Fig 4 the values of the various f I slopes are plotted against time to peak of late afterdepolarization In Fig 5 dashed areas signify motoneurones with a time to peak of late afterdepolarization longer than 90 msec

Sasaki and Otani (1961 1962) suggested on the basis of their experiments on accommodation that motoneurones with short afterhyperpolarization (mean duration 72 msec) generally would require a higher current strength (in units of rheobase) to fire repetitively than motoneurones with long afterhyperpolarization (mean duration 102 msec), at least in cats anaesthetized by Nembutal This could not be confirmed in the present experiments The threshold for continuous firing varied between 1 7—33 0 nA and was on the average 11 5±9 2 (S D , 50 cells)nA In units of rheobase the threshold for continuous firing was 1 5 ± 0 4 (S D , 43 cells), which is close to the corresponding value obtained from rat motoneurones (Granit *et al* 1963 *a*) For 17 units with time to peak of late afterdepolarization shorter than 70 msec the relation between the threshold for continuous firing and the rheobase was 1 5 ± 0 4 (S D), for 11 units with time to peak of late afterdepolarization longer than 100 msec, it was 1 7 ± 0 6 (S D) The difference was not significant (t test, $p > 0.2$) The threshold for continuous firing was always higher than the rheobase

The findings described above concerning the variation in adaptation, f I slopes and the threshold for continuous firing remained valid also when cells with high spike amplitudes or membrane potentials were selected

Discussion

Granit *et al* (1963 *a* *b*) studied the continuous repetitive firing of rat and cat motoneurones which were stimulated by long lasting injected currents Their findings concerning continuous discharges could, however, not be immediately applied to the repetitive firing of cat motoneurones because most of their results were obtained from the rat, and also because several of their cells discharged only short bursts in response to steady currents It appears from the present findings that many of the transient responses were from cells damaged by the microelectrode and hence excessively depolarized, and

that at least the majority of undamaged cat motoneurones would be capable of setting up a continuous repetitive discharge in response to a long lasting current (*cf* Pascoe 1957) For repetitive firing set up by steady reflex stimulation this implies that a motoneurone need only be excited strongly enough in order to respond by a continuous discharge (*cf* Henatsch, Schulte and Busch 1959) The present data concerning the threshold for continuous firing the f I relation and the adaptation of continuously discharging cat motoneurones might also be helpful in the analysis of reflex firing The quantitative data were on the whole well in line with those of Granit *et al* (1963 *a* *b*) Some special problems concerning repetitive firing at high frequencies and the limits of discharge frequency will be treated in two consecutive papers (Kernell 1965 *a* *b*)

The existence of inherently phasic cells is hard to exclude in the present complicated situation (*cf* also Barron and Matthews 1938, Fuortes 1954), but at least they appear to be comparatively scarce This view is consistent with the finding that motoneurones when tested by slowly rising currents usually show but little accommodation (Frank and Fuortes 1960 Bradley and Somjen 1961, Sasaki and Otani 1961 1962) Minimal current gradients have generally been found only in motoneurones with low spike amplitudes or during signs of deterioration (Bradley and Somjen 1961, Sasaki and Otani 1962)

It is in this context of interest to note that also the pyramidal neurones of cat cerebral cortex generally are capable of responding by maintained repetitive firing to a steady injected current (Creutzfeldt, Lux and Nacimiento 1964) The firing rate decreased during the initial 70—100 msec, but was thereafter virtually constant The f I relation was approximately linear also in the pyramidal cells The rhythmic properties of these cortical neurones are thus similar to those of continuously firing motoneurones as determined in the present investigation To judge by the illustrations of Creutzfeldt *et al* (1964 the steady state f I slope has a much larger absolute value (in imp sec nA) in the pyramidal cells than in lumbosacral motoneurones The two types of neurones appear to differ also in that the early drop of firing rate in the pyramidal cells occurs to a large extent without a concomitant decrease of the f I slope

The durations of afterhyperpolarization i e time to peak of late afterdepolarization encountered in the present material were usually between 50—120 msec (Fig 2) and practically always shorter than the durations of afterhyperpolarization of soleus motoneurones as measured by Eccles *et al* (1958 1961) Such properties of repetitive firing which varied with the duration of afterhyperpolarization in a continuous manner would however presumably be revealed also by the present material The results strongly indicate that the majority of undamaged cat lumbosacral motoneurones whatever their duration of afterhyperpolarization (*cf* Fig 2) are capable of producing a well maintained repetitive discharge in response to a steady current Very little adaptation and a low threshold for continuous firing (in units of rheobase cf Sasaki and Otani 1961 1962 was also found in many motoneurones with very short time to peak of late afterdepolarization The only differences between the repetitive discharges of motoneurones with different durations of afterhyperpolarization that could be found by the present method concern the upper and lower limits of firing frequency but these will be considered in a subsequent paper Kernell 1965 *b* Thus the present findings favour the view that the differences between tonic and phasic motoneurones in their capacity to fire repetitively in response to muscle stretch (see Introduction are mainly due to differences of synaptic organization such as those described by Eccles *et al* (1957) For a final test of this point it would be important to compare effects of muscle stretch with those of injected currents in the same motoneurones

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Acta physiol. scand. 1965. 65. 74—86

From the Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden

High-Frequency Repetitive Firing of Cat Lumbosacral Motoneurones Stimulated by Long-Lasting Injected Currents

By

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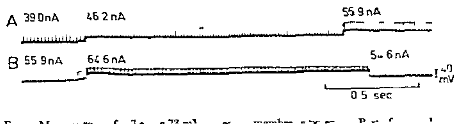
Abstract

Kernell, D. *High-frequency repetitive firing of cat lumbosacral motoneurones stimulated by long-lasting injected currents.* Acta physiol. scand. 1965. 65. 74—86. Repetitive discharges were initiated in motoneurones by steady currents injected through an intracellular microelectrode. At weaker currents, producing firing rates up to on the average 51 (30—81) imp/sec, [illegible]

each approximately fitted by a separate straight line in the graphs relating impulse frequency to current strength. The straight line which referred to steady firing within the secondary range had a 2—6 times steeper slope than the one characterizing the primary range. Motoneurones [illegible]

to the repetitive firing of reflexly activated motoneurones.

Granit, Kernell and Shortess (1963 a, b) studied with an intracellular recording technique the repetitive firing of motoneurones stimulated by long-lasting injected currents. They found that in steady repetitive firing (by them called "adapted" firing) the relation between discharge frequency and current strength was well fitted by a straight line up to current strengths producing firing rates on the average of 64 imp/sec in rat and 37 imp/sec in cat motoneurones. At higher current strengths, the curves tended to decline. Very much higher discharge frequencies could, however, be obtained from the early portion of the repetitive discharge. During the initial phase of "adaptation" the impulse frequency diminished at a rate which varied from cell to cell and was dependent on current strength. Also during the initial portion of the repetitive discharge the firing



[illegible]

rate was approximately linearly related to current strength, but the slope of this linear curve was considerably steeper than that obtained for the subsequent steady state of firing

Most of the investigations of Granit, Kernell and Shortess (1963 *a*) were performed on rat motoneurones When trying now to extend this work to a larger material of cat motoneurones it proved possible in many cells to elicit comparatively high frequencies of steady discharge by injected currents In plotting the discharge rates against current strengths the values obtained with the strongest currents very obviously fell above the straight line that fitted points for weaker currents In such cells the curve relating steady firing rate to current strength had a steeper slope for strong than for weak currents For the initial spike intervals of the repetitive discharge the corresponding curves generally showed upward deflections with stronger currents and this held good also for cells in which the curves for later periods of discharge turned downward. A description of these findings forms the subject of the present paper The findings are discussed also in relation to problems concerning the repetitive firing elicited by reflex activation

Methods

Most of the present results were derived from the same experiments as those of an earlier paper (Kernell 1965 *a*) in which the methods were given in detail The experiments were performed [illegible]

The present investigation is concerned only with motoneurones which were capable of responding to stimulation by long lasting depolarizing currents with a well maintained repetitive discharge (*cf* Fig 1 and 6A) Some cells were stimulated by constant currents generally lasting 2 sec or more and a pause of about half a minute was allowed between each period of stimulation (*cf* Fig 6) Other neurones were continuously discharged for about 10—60 sec while the strength of current was changed in steps (*cf* Fig 1) At least over a range of weaker current strengths these two methods give practically equal values for the relation between steady firing rate and current strength (Granit Kernell and Shortess 1963 *b* Kernell 1965 *a* Only the former method can be used for investigations of the relation between firing rate and discharge frequency during the initial spike intervals of repetitive discharges

Many cells which showed well maintained repetitive firing in response to injected currents could not be used in the present investigations either because their membrane potential changed during the testing procedure or because they deteriorated or were lost before a sufficient number of measurements could be obtained

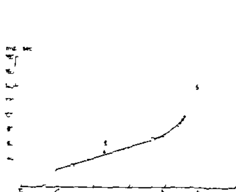


Fig 2

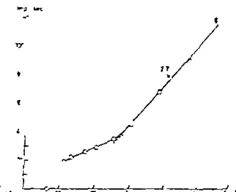


Fig 3

Fig 2 Same cell as Fig 1 Steady discharge frequencies plotted against current strength. The values were obtained from the discharge which is partly shown in Fig 1 Current strength was changed in steps at 1 0—1 5 sec intervals and firing rates were measured over the final 0 5 sec of discharge at each current strength Straight lines are fitted to the values obtained at weak and strong currents respectively and the slopes of these lines (imp sec nA) are indicated by numbers at arrows.

Fig 3 Motoneurone of spike size 93 mV at resting membrane potential Steady discharge frequencies are plotted against current strength and straight lines are fitted to the values as in Fig 2 The values were obtained from a continuous discharge (cf Fig 1) and firing rates were measured at 0 1—0 6 sec following the onset of each current strength

Firing rates which on the whole do not decrease or increase over at least half a second of constant stimulation will be referred to as *'steady discharge frequencies' The lowest current* strength which is capable of eliciting a well maintained repetitive discharge will be called the threshold for continuous firing The relation between discharge frequency (f) and current strength I) will be called the f I relation Current strength is expressed in nA (1 nA = 10^{-9} A

Results

Steady repetitive firing

Fig 1 shows records from a cell which would discharge to injected currents in a maintained manner up to very high rates The cell was continuously stimulated by a long lasting injected current whose strength was changed in steps as indicated Fig 1) During the initial 0 1 sec after onset of the current at strength 64 6 nA Fig 1 B the firing rate averaged 210 imp sec One sec after the onset of this current strength the discharge frequency proved to be stable at around 170 imp sec Fig 1 also shows that both before and after stimulation by 64 6 nA, steady firing at around 100 imp sec was obtained at current strengths 55 9 and 54 6 nA

In Fig 2 steady discharge frequencies are plotted against the respective strengths of injected current The values were obtained from the long lasting discharge part of which is shown in Fig 1 At current strengths of 46 2 nA or less the values are well fitted by a straight line with a slope of around 1 6 imp sec nA For stronger currents the curve relating discharge frequency to current strength the f I curve bends off upwards Fig 2 A similar upward deflection of the f I curve for steady firing was seen in 14 out of 30 investigated motoneurones

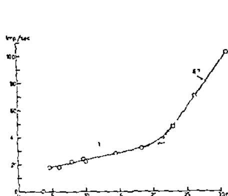


Fig 4

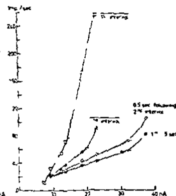


Fig 5

In Fig 2 the values are too few to show the shape of the steeper part of the f I curve A greater number of values were obtained from six other motoneurones behaving similarly, and two of these are illustrated by Figs 3 and 4 The highest discharge frequencies which are plotted in these two diagrams (Figs 3 and 4) correspond to the maximal rates of steady firing which could be elicited by injected currents in the respective experiments In some cells the f-I curve showed a tendency to become less steep at the very highest firing rates However, it appears from these six cells that the relation between steady discharge frequency and current strength could be approximately described by two straight lines of different slopes (Figs 3 and 4) One of these lines extends over a range from the threshold for continuous firing and upwards (Figs 3 and 4) and its slope will in the following be referred to as "primary f I slope" The corresponding discharges will be said to occur within the "primary range" of firing Discharges at frequencies higher than those compatible with a primary f I slope will be referred to as firing within the "secondary range", and the slope as the "secondary f-I slope" All f I slopes (slope constants) are given in imp/sec/nA

In the six cells with which a large enough number of measurements was obtained for showing that the f I curve for steady firing was approximately linear also in the secondary range the values of primary and secondary f I slopes (the latter within brackets) were 1 1 (2 7) (Fig 3), 1 1 (6 7) (Fig 4) 1 4 (3 5) 2 3 (4 4), 2 0 (4 0) 2 5 (5 0) imp sec/nA In three other motoneurones only two measurements were available for an approximate calculation of the secondary f I slopes The primary and secondary f-I slopes for steady repetitive firing in these cells were 1 6 (7 5) (Fig 2) 1 7 (4 0), 2 0 (4 0) imp/sec/nA

On an average the primary *f*-*I* slope for these nine cells was 1.7 imp/sec/nA and the corresponding secondary *f*-*I* slope averaged 4.6 imp/sec/nA (Fig. 8). The values show that about 2—6 times less current is needed to obtain a given change in steady discharge frequency at high than at low current strengths in the cells which are capable of discharging in a maintained manner in the secondary range. Such cells are also capable of setting up a steady discharge of very high firing rate (Fig. 1). For nine motoneurones whose *f*-*I* slopes are given above, the maximal frequency of steady discharge averaged 125 ± 38 (88—195) imp/sec (mean, S.D. and range). These maximal firing rates were produced by current strengths 4.2 ± 1.4 (2.4—6.6) times the threshold for continuous firing. It should be pointed out that in most of the cells comparatively few current strengths were used so that the values given for secondary *f*-*I* slopes and maximal frequencies of steady discharge in the secondary range are only approximations indicating order of magnitude.

In the present material of 30 motoneurones 14 were capable of setting up steady discharges in the secondary range. In them the primary *f*-*I* slope for steady repetitive firing was on the average valid up to a current strength of 2.7 ± 0.7 (1.6—4.2) times the threshold for continuous firing. The maximal frequency of steady firing within the primary range was 52 ± 17 (30—74) imp/sec. In 5 cells the maximal discharge frequencies within the range of the primary *f*-*I* slope were as low as 30—36 imp/sec (*cf.* Figs. 3 and 4).

Discharges at frequencies within the secondary range could be well maintained at least during many seconds of continuous stimulation (up to 19.5 sec tested in one cell). With weak currents the same cells always had a primary *f*-*I* slope both immediately before and after the application of the stronger current by which firing rates within the secondary range were determined.

In 30 motoneurones there were 16 cells which would show steady discharges practically only within the range of the primary *f*-*I* slope, i.e. the *f*-*I* relation for steady firing could in each of these 16 cells be approximately described by a single straight line (*cf.* Fig. 7). With stronger currents firing was not well maintained and the *f*-*I* curve ultimately took a downward course (*cf.* Figs. 6 and 7). These 16 cells thus behaved like those described by Granit, Kernell and Shortess (1963 *a*). The fitting of straight lines to the *f*-*I* relation for steady firing within the primary range could for them be carried up to a current strength of 2.8 ± 1.1 (1.3—5.4) times the threshold for continuous firing. The maximal frequency of steady firing within the primary range was 51 ± 13 (30—84) imp/sec. These averages as well as the range of variation of individual values are very similar to the corresponding values for the 14 cells capable of setting up steady discharges also in the secondary range (see above). For all the 30 motoneurones taken together the maximal current strength with which steady firing occurred within the primary range averaged 2.7 ± 1.0 (1.3—5.4) times the threshold for continuous firing. The corresponding maximum frequency of steady firing within the primary range was 51 ± 15 (30—84) imp/sec. The motoneurones which did and those which did not maintain firing within the secondary range showed no statistically significant differences with respect to their average value of the primary *f*-*I* slope, the time course of afterhyperpolarization, the spike amplitude and the threshold for continuous firing. Within both groups there were units possessing spike amplitudes up to 92 mV and resting membrane potentials up to 74 mV. However, it was seen in a few cases that discharges in the secondary range were better maintained when the membrane potential in the course of an experiment spontaneously increased. These observations indicate that some moto-

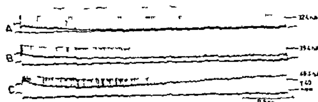


Fig 6 Motoneurone of spike size 82 mV at resting membrane potential. Repetitive discharges set up by the current strengths indicated A pause (about 30 sec) was allowed between each strength of stimulation During the initial part of C the peaks of the spikes are not shown The upper beam signals strength of current

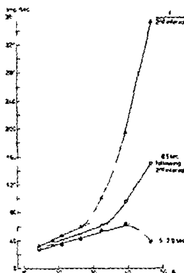
neurones might have been less capable of maintaining discharges in the secondary range because they were damaged by the micro-electrode, and hence abnormally depolarized

Adaptation

The curves of Fig 5 were obtained from a motoneurone which was capable of steady firing in the secondary range Impulse frequencies are plotted against current strengths for the first and the second spike interval as well as for later periods of the discharge For each current strength the firing rate is higher in the early than in the later parts of the discharge (Fig 5) The decrease of firing rate as a function of stimulus duration will be referred to as 'adaptation' (Granit, Kernell and Shortess 1963 *a*) It is seen in Fig 5 that the respective values are well fitted by straight lines over a range of weaker currents With stronger currents the f-I curves are deflected upwards for the initial intervals as well as for later periods Thus the subdivision of maintained firing into "primary" and "secondary" range can apparently also be used for the earliest intervals of the repetitive discharge The primary f I slope has a larger value initially than later on in repetitive firing to trans-membrane currents (Fig 5) as shown by Granit, Kernell and Shortess (1963 *a* *cf* also Shapovalov 1964, Kernell 1965 *a*)

In 8 of the motoneurones which were capable of steady firing in the secondary range, the relation between impulse frequency and current strength was also investigated for the initial intervals of the repetitive discharge In all these cases the curves for the initial intervals became deflected upwards with stronger currents (*cf* Fig 5)

The values plotted in Fig 7 are from a motoneurone which did not maintain firing within the secondary range Some of the records evaluated in Fig 7 are shown in Fig 6 With the strongest currents, the f I curve in the end turns downwards (Fig 7), and at this strength steady firing no more occurs (Fig 6 C) However the curves from earlier parts of the repetitive discharge are deflected upwards (Fig 7) Apparently the cell is capable of firing in the secondary range for a brief time but in the long run cannot maintain these high discharge rates The initial f I relation could be measured in 12 out of the 16 motoneurones incapable of maintained firing in the secondary range Of these cells 6 fired transiently in the secondary range for about 0 5—1 0 sec after the onset of stimulation (Fig 7) In the other 6 cells, firing in the secondary range lasted for brief durations sometimes permitting a few spikes only It appears from the present results that fundamentally all motoneurones are capable of discharging in the secondary range but some of them only for extremely brief durations



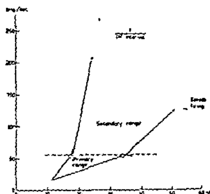


Fig 7 Fig 8

Fig 7 Same cell as Fig 6 Diagram showing the relation between discharge frequency and current strength at the indicated times after onset of stimulation. Three of the discharges from which the measurements were obtained are shown in Fig 6 Straight lines are fitted to the values obtained at weaker currents, and the slopes of these lines (the primary f I slopes) are 2 2, 1 8 and 1 4 imp sec nA respectively

Fig 8 Schematic Comparison of the relation between firing rate and current strength at weak and at strong currents The lines represent the first impulse interval after onset of current and the steady repetitive firing respectively The diagram is based on average values obtained from different cells In the diagram the values of primary and secondary f I slopes for the first impulse interval are 6 5 and 24 9 imp sec/nA respectively (averages from 10 cells) The values of primary and secondary f I slopes for steady firing are 1 7 and 4 6 imp sec nA respectively (averages from 9 cells The threshold for continuous firing is 11 5 nA (average from 50 cells Kernell 1965 a The values of maximal current strength and discharge frequency within the primary range are taken from Table 1 (marked by circles in the diagram) The approximate border between the primary and the secondary range is indicated by the horizontal interrupted line Further comments in text

In Fig 5 and 7 it is seen that the linear curves within the primary range change their slope at approximately the same firing rate fairly independent of stimulus duration This behaviour recurred in the 20 motoneurones investigated, the maximal discharge frequencies obtained within the primary range could be higher initially or later, in a random manner but large discrepancies were but rarely encountered The discrepancies that occurred may often have been due to the fact that the full extension of the primary range in most cases had to be approximated on the basis of a relatively small number of current steps As the cells adapted the maximal discharge frequency within the primary range required increasingly stronger trans-membrane stimulation The above results held equally good for neurones which did (Fig 5 as well as for those which did not (Fig 7 discharge steadily within the secondary range Thus the diminution of the slope within the primary range parallels an extension of the range itself in terms of current strength Table 1 shows how well in experiments on adaptation the upper limit of the primary

TABLE I The maximal current strength and discharge frequency within the range of primary f I slopes from different parts of repetive discharges

	First interval				Second interval				Steady repetitive firing			
	Mean	Range	S D	n	Mean	Range	S D	n	Mean	Range	S D	n
Current strength (in units of threshold for continuous firing	1 5	1 0—2 7	±0 5	13	2 1	1.5—2 8	±0 4	13	3 0	1 8—4 5	±0 8	13
Discharge frequency (imp/sec)	54	22—83	±20	13	55	16—100	±25	13	54	30—74	±14	13

All values are from the same 13 motoneurones S D = standard deviation n = number of cells Differences between the average values of discharge frequency are not statistically significant (t test p 0 9) Differences between the average values of current strength for the first and the second interval and for the second interval and steady repetitive firing respectively are statistically significant (t test $0\ 01 > p > 0\ 001$) The material consists of 6 cells which did and 7 cells which did not discharge steadily in the secondary range Essentially similar results were obtained as the data from each of the two groups were compared separately

range is set by a constant maximal impulse frequency rather than by constant current This is not the case in the secondary range in which discharge frequencies as high as 300—600 imp/sec only occur at the earliest spike intervals succeeding onset of stimulation

In the secondary range, the impulse frequency was often related to current strength in a roughly linear fashion also for firing rates measured from the first two intervals of the repetitive discharge The corresponding curves generally became less steep at firing [illegible]

300 imp sec the primary and secondary f I slopes for the first interval of the repetitive discharges were 6 5 ± 3 2 and 24 9 ± 9 7 imp/sec/nA respectively (mean and S D for 10 cells) It appears that for firing in the secondary range, similar to what has been shown for firing within the primary range the slope of the f I curve is much steeper initially than later on in the discharge (*cf* Fig 5 and 7)

The main findings of the present study are schematically presented in Fig 8, which shows the approximate relation between firing rate and current strength for the first spike interval and for steady firing respectively The diagram was constructed on the basis of average values obtained from different cells The numerical values of discharge frequencies current strengths and f I slopes differ considerably from cell to cell, but the values for each cell were interrelated in a manner similar to Fig 8

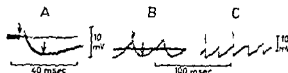


Fig 9 Same cell as Fig 3 Records illustrating the time course of the afterpotentials by antidrom c

Afterpotentials

As was described above, motoneurones have different repetitive properties when stimulated by weak and strong currents Signs of a change in the behaviour of a cell above a certain current strength were found also in the afterpotentials succeeding the spikes From observing the afterpotentials succeeding the repetitive spikes it was generally possible to predict whether any given discharge fell within the primary range or not

As long as strength of current was low, the motoneurones which would set up well maintained discharges always fired repetitive spikes succeeded by afterpotentials ... d at all ... B it is ... latively long lasting phase of progressive increase of membrane potential The time from the onset of a spike potential to the point at which it reaches its maximum value (Fig 9 A and B arrows) will be referred to as time to maximal afterhyperpolarization With antidromic spikes (cf Fig 9 A) the time to maximal afterhyperpolarization of 13 motoneurones was on the average 14 5 (9—23) msec With repetitive spikes at the lowest firing rate the corresponding value was 13 4 (8—21) msec, and at the maximum firing rate within the primary range 10 3 (6—19) msec

In discharges elicited by currents stronger than those which set up firing within the primary range the time to maximal afterhyperpolarization was practically always very short around 2 msec) and the repetitive spikes were succeeded by afterpotentials similar to those shown in Fig 9 C This held good irrespectively of whether firing could be maintained at frequencies within the secondary range or not As has been shown certain current strengths would initially elicit firing within the secondary range and later on only within the primary range (Figs 5 and 7) In such cases the initial impulses had a very short time to maximal afterhyperpolarization (around 2 msec) When later on firing occurred only within the primary range the spikes had afterpotentials with a long time to maximal afterhyperpolarization (around 6—19 msec During slow transitions from initial firing within the secondary range to later firing within the primary range (cf Fig 6 and 7 the time to maximal afterhyperpolarization often varied in an irregular manner being sometimes long sometimes short

The size of the repetitive spikes measured from the discharge level to the peak of the action potential generally became smaller as firing frequency increased and this held good also within the primary range There was however no especially marked change in the spike size at the turning point from the primary to the secondary range The

transition from initial firing within the secondary range to later firing within the primary range was generally not accompanied by an increased spike size (*cf* Fig 6 and 7) Firing within the secondary range, accompanied by short time to maximal afterhyperpolarization (around 2 msec), could often by seen with repetitive spikes which were around 50—60 mV, and thus definitely above the size of so-called IS or A spikes (Coombs Curtis and Eccles 1957, Fuortes, Frank and Becker 1957) Also in the repetitive discharges of reflexly activated motoneurones the spike size has been found to decrease as the firing rate increases (Kolmodin and Skoglund 1958)

Many cells did not respond by any recurrent inhibitory post synaptic potential (Eccles Fatt and Koketsu 1954, Eccles *et al* 1961) to antidromic stimulation At the end of a penetration several such cells became incapable of delivering an antidromic spike When this happened the orthodromic post-synaptic potentials would still be present, and so the lack or presence of recurrent inhibitory post synaptic potentials could be checked by antidromic stimulation in the absence of spike responses The general behaviour of the f I relation and the afterpotentials in repetitive discharges to injected currents was the same in motoneurones with and without signs of recurrent inhibition

Discussion

Granit Kernell and Shortess (1963 *a*) did not find motoneurones which were capable of setting up steady firing in the secondary range However most of their experiments were performed on rats Even at low current strengths they found it more difficult to produce well maintained repetitive discharges in motoneurones from rats than in those from cats Rat motoneurones are on the whole smaller than cat motoneurones (Granit Kernell and Smith 1963) hence they are presumably more easily damaged by the micro-electrode [illegible]

Extensive investigations of the relation between firing rate and current strength have been performed with the pyramidal cells of cat s cerebral cortex (Creutzfeldt *et al* 1964) Also in these neurones the slope of the f I curve was steeper with strong than with weak currents for the initial part of the repetitive discharge In steady firing the curves were [illegible]

found to be [illegible]

bu [illegible]

T [illegible]

from cat motoneurones (*cf* Figs 3 and 4)

Quantitative descriptions of the repetitive firing of motoneurones in response to injected currents as given in previous papers Granit Kernell and Shortess 1963 *a* *b* Shapovalov 1964 Kernell 1965 *a*) and in the present investigation (*cf* Fig 8) should aid us in the understanding of repetitive firing in response to reflex activation see below It is however difficult at the present time to give a good theoretical explanation of the facts presented by the above investigations because relatively little is known about the membrane properties of motoneurones and the complicated mechanisms taking part in the production of a repetitive discharge The marked changes seen in the properties of

motoneurones with regard to slope of f I curves adaptation (Fig 8) and afterpotentials (Fig 9) dependent on current strength and duration may be, for instance, due to a shift in the site of impulse initiation or to a block of some portion of the membrane adjacent to the micro-electrode Firing in the secondary range did presumably occur without a complete block of the membrane responsible for the SD or B-component of the spike potential (Coombs *et al* 1957, Fuortes *et al* 1957), because the spikes often considerably exceeded the IS or A-component in size (p 10) In the experiments on *adaptation it was found that the maximal firing rate within the primary range remained* about the same although less current was needed for producing maximum rates initially than later on (Figs 5, 7 and 8) These results indicate that not only the current strength but also the repetition rate could be of importance in determining the upper limit of the primary range Recurrent inhibition (Eccles *et al* 1954, 1961) appears not to be decisive in the present context (p 10) It is also difficult to attribute the changed properties of the motoneurones with stronger current (*cf* Figs 8 and 9) to an intracellular accumulation of ions introduced from the micro-electrode because the cells fired optimally in the secondary range (Fig 8) with a very short time to maximal afterhyperpolarization (Fig 9 C) *just after the onset of the stimulating current* It is not clear why some cells would only set up transient discharges in the secondary range Some of these latter cells might have been damaged by the micro-electrode (p 5)

The afterpotentials ocurring in repetitive discharges as well as problems concerning adaptation, have recently been discussed also by Granit Kernell and Shortess (1963 *a*) and Granit, Kernell and Smith (1963) A further discussion has to wait until more information is available

Earlier studies have strongly indicated that the repetitive firing of motoneurones in response to maintained reflex activation is set up chiefly by relatively steady synaptic currents (e g Eccles 1953, p 174 ff 1964, p 114 ff, Frank and Fuortes 1961, Granit and Renkin 1961) Results obtained by injected currents should clearly be tested also in situations employing synaptic excitation The high slopes of the f I curves in the secondary range suggest however, that at higher firing rates the discharge frequency of reflexly activated motoneurones can be influenced by fairly small changes in the synaptic input *cf* Fig 8) With inhibition for instance, the same stimulus would cause a much larger decrease of firing rate at high than at low discharge frequencies Nothing of this sort was found by Granit and Renkin (1961), who studied the interaction between synaptic excitation and recurrent inhibition in repetitively discharging motoneurones However their discharge frequencies were generally less than 40 imp/sec and at those rates the secondary range of firing would not be of much importance It is therefore of interest to note that at least some lumbosacral alpha motoneurones may discharge at high rates also in response to synaptic excitation (e g Adrian and Bronk 1929 Wilson and Talbot 1964 Motoneurones belonging to the quadriceps muscle are known to reach firing rates as high as 60 90 imp sec during long lasting discharges elicited by reflex stimulation Adrian and Bronk 1929 Even in the anaesthetized animals used in the present investigation it was possible by tetanic stimulation of the brain stem or of the hamstring or the popliteal nerves to elicit maintained discharges in some moto- [illegible] ... rones may [illegible]

For a further discussion of the possible physiological significance of the present [illegible] it would be of interest to know whether motoneurones innervating fast muscles start to

discharge in the secondary range at higher firing rates than motoneurones belonging to slow muscles Antidromic spikes are generally succeeded by short afterhyperpolarizations in motoneurones innervating fast-contracting muscles (Eccles, Eccles and Lundberg 1958 Kuno 1959, Eccles *et al* 1961) In a subsequent paper it will be investigated whether the maximal discharge frequency in the primary range is correlated with the time course of the afterhyperpolarization succeeding antidromic spikes (Kernell 1965 *b*)

Under the present experimental conditions it was generally possible to predict from the time course of the afterpotentials (*cf* Fig 9), whether a steady discharge belonged to the primary or the secondary range These findings suggest that also in the repetitive discharge elicited by synaptic activation, the time course of the afterpotentials may re

[illegible]

frequencies (Kernell unpublished) In repetitive discharges elicited by muscle stretch the afterhyperpolarizations' are often much less prominent at higher than at lower firing rates (Kolmodin and Skoglund 1958, Granit, Kellerth and Williams 1964) In such discharges the time course of the afterpotentials measured as time to maximal afterhyperpolarization becomes also progressively shorter at higher firing rates (Kolmodin and Skoglund 1958)

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The Limits of Firing Frequency in Cat Lumbosacral Motoneurones Possessing Different Time Course of Afterhyperpolarization

By

Daniel Kernell

Received 18 January 1965

Abstract

Kernell D. *The limits of firing frequency in cat lumbosacral motoneurones possessing different time course of* ... Acta physiol scand 1965 65 87—100 — Long lasting repetitive

of the motoneurones

In experiments employing various forms of 'natural' stimulation it has been observed that the firing rates are often higher in motoneurones innervating rapidly contracting muscles than in those belonging to slowly contracting ones (e g Adrian and Bronk 1929, Denny-Brown 1929, Reid 1949, Sasaki 1963) The antidromic spikes of cat motoneurones

of their inherent properties discharge repetitively at faster frequencies than the cells

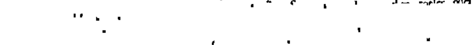
repetitive firing of rat and cat motoneurones stimulated by injected currents The discharge frequencies were on the whole higher in rat motoneurones whose antidromic spike discharges generally possess shorter afterhyperpolarizations than have those of cat motoneurones (Bradley and Somjen 1961)

The aim of the present study was to compare the discharge frequencies initiated by injected currents in cat motoneurones which had afterhyperpolarizations of different durations, as measured by antidromic spike discharges The firing rates of these motoneurones will partly be considered in conjunction with the relation between discharge frequency (f) and current strength (I) (the 'f I relation) in the respective cells The proportionality constant of the linear f I relation will be called the f I slope' The f I slope of that part of the f I relation which is linear for firing rates down to the minimal discharge frequency will be referred to as the primary f I slope' (Granit *et al* 1963 Kernell 1965 *b*) Firing rates for which the f I relation is linear with a primary f I slope will be referred to as discharge frequencies in the primary range' Firing rates which are higher than what is compatible with a primary f I slope will be referred to as discharge frequencies in the secondary range (Kernell 1965 *b*) The f I relation is often approximately linear also for most of the firing frequencies in the secondary range and the value of the secondary f I slope is always larger than that of the corresponding primary f I slope from the same cell (Kernell 1965 *b*) Many motoneurones are not capable of maintaining discharges in the secondary range of firing (Granit *et al* 1963 Kernell 1965 *b*)

It will be shown in the present work that in long lasting repetitive discharges the minimal firing frequency, the maximal firing frequency in the primary range and the maximal firing frequency in the secondary range are all faster in motoneurones which have short afterhyperpolarizations following their antidromic spikes The findings will be discussed mainly in relation to problems concerning control of muscle tension by motoneurones

Methods

Most of the results were derived from the same experiments as those described in two earlier papers (Kernell 1965 *a b*) and the methods were given by Kernell (1965 *a*) The experiments were performed on cats anesthetized by pentobarbitone (35—40 mg kg i p) Intracellular



similar to that shown in Fig 2 C

Current strength is expressed in nA (1 nA = 10 ⁹ A) Discharge frequencies are given in impulses per second (imp sec

Results

The time course of afterpotentials succeeding antidromic spikes

In the present investigation discharge frequencies were related to the time course of afterpotentials succeeding antidromic spike discharges the latter being elicited at resting membrane potential The afterpotentials were measured in two different ways either from the onset of a spike discharge to the point of maximal afterhyperpolarization ("time to maximal afterhyperpolarization between the lower arrows in the inset

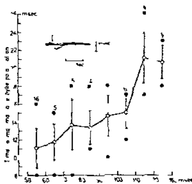
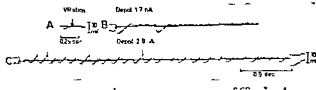


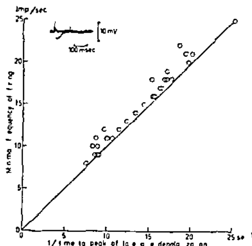
Fig 1 Diagram showing the relation between time to maximal afterhyperpolarization and time to peak of late afterdepolarization in 90 motoneurones Spike discharges were elicited by antidromic stimulation and the afterpotentials were measured as indicated by arrows in the inset The population was arbitrarily grouped into classes at 10 msec intervals along the abscissa, except for the last class which was made larger to increase the size of the sample Vertical solid lines indicate the sizes of the respective standard deviations The range of variation of values within each class is indicated by filled circles Mean values are for each class indicated by circles *Inset* records from threshold antidromic stimulation of motoneurone 15—20 superimposed sweeps



signals strength of current

of Fig 1), or from the onset of the spike to the peak of the late afterdepolarization ("time to peak of late afterdepolarization", between the upper arrows in the inset of Fig 1) Time to peak of late afterdepolarization corresponds to the duration of afterhyperpolarization as measured by Eccles *et al* (1958) Time to maximal afterhyperpolarization has previously been measured only sporadically (e g Brock, Coombs and Eccles 1952) From earlier experiments it would be expected that the time to maximal afterhyperpolarization is significantly correlated to the time to peak of late afterdepolarization (*cf* Eccles *et al* 1958, Kuno 1959) From the point of view of the present work it is, however, of some interest to know how closely these measurements correlate

Fig 1 shows that the relation between time to maximal afterhyperpolarization and time to peak of late afterdepolarization actually is rather variable The same value of time to maximal afterhyperpolarization could be obtained from motoneurones with any time to peak of late afterdepolarization less than 110 msec (Fig 1) The diagram also indicates that the values of time to maximal afterhyperpolarization obtained from cells with time to peak of late afterdepolarization shorter and longer than 110 msec respectively show practically no overlap (Fig 1) The number of cells with a long time to peak of late afterdepolarization is, however, too small to allow any definite conclusions on this point The relation between time to maximal afterhyperpolarization and time to peak of late afterdepolarization had a correlation coefficient r = + 0 76 for the 90 cells of Fig 1 When considering only the 77 cells which had a time to peak of late afterdepolarization shorter than 110 msec (Fig 1) the correlation coefficient was +0 51 The correlation was highly significant in both cases (t test, 0 001 > p)



[illegible] motoneurones Firing rates were measured over 0 5 sec in long lasting discharges elicited by injected currents Time to peak of late afterdepolarization was measured as indicated by arrows in the inset The line is drawn through points for which the minimal firing frequency = 1/time to peak of late afterdepolarization *Inset* record from threshold antidromic stimulation of motoneurone 15—20 superimposed sweeps

The spread of the values in Fig 1 could not have been caused only by errors of measurement In the majority of cases time to peak of late afterdepolarization could easily be approximated to within a few milliseconds, and time to maximal afterhyperpolarization could be measured to within a millisecond

The variable relation between time to maximal afterhyperpolarization and time to peak of late afterdepolarization is apparently not due to the varying amounts of recurrent inhibition impinging upon the cells Of the 90 motoneurones in Fig 1, 53 showed no recurrent inhibitory post synaptic potential (Eccles, Fatt and Koketsu 1954) when stimulated antidromically just at threshold for eliciting a spike response (*cf* Fig 2A) A curve which was almost identical with that of Fig 1, and which showed the same spread of values was obtained from these 53 cells alone

Both time to maximal afterhyperpolarization and time to peak of late afterdepolarization were always virtually the same after spikes elicited by short injected pulses of current as those following after antidromic spikes This was also the case in units showing recurrent inhibitory post synaptic potentials of moderate size to antidromic stimulation In two of the cells which were used for the analysis of repetitive firing (see below), the antidromic spikes had become blocked, and in these two cases the time course of the afterpotentials was actually measured from single spike discharges elicited by depolarizing pulses of less than 1 msec in duration In all the other cells the time course of the afterpotentials was measured from antidromic spike discharges

Minimal firing frequencies

In the vast majority of cat motoneurones the minimum frequency of continuous firing could be precisely defined and measured With steady repetitive discharges at the minimal firing rate a slight lowering of the current strength would either make the repetitive firing highly irregular or the repetitive discharge would stop altogether (*cf* Granit *et al* 1963

In most of the 31 motoneurones whose minimal firing frequencies were investigated the late afterdepolarization which succeeds the afterhyperpolarization appeared to be larger after spikes in irregular repetitive discharges set up by weak currents (Fig 2B than after spikes elicited antidromically at the resting membrane potential (Fig 2A

TABLE I Correlations between limits of discharge frequency and reciprocal values of the time course of afterpotentials

	r	n	P	
Minimal discharge frequency and A (cf Fig 3)	+ 0 98	31	0 001>p	Highly significant
Minimal discharge frequency and B	+ 0 74	31	0 001>p	Highly significant
Maximal discharge frequency in primary range and A (cf Fig 4)	+ 0 58	32	0 001>p	Highly significant
Maximal discharge frequency in primary range and B (cf Fig 5)	+ 0 73	32	0 001>p	Highly significant
Maximal discharge frequency in secondary range and A (cf Fig 7)	+ 0 69	13	0 01>p>0 001	Significant
Maximal discharge frequency in secondary range and B	+ 0 76	13	0 01>p>0 001	Significant

r = correlation coefficient n = size of the sample (number of cells) P = probability
A = 1/time to peak of late afterdepolarization
B = 1/time to maximal afterhyperpolarization

arrow) The time to peak of late afterdepolarization was, however, much the same after spikes in irregular repetitive discharges set up by weak currents (Fig 2B) as after those elicited antidromically at the resting membrane potential (Fig 2A, arrow) As a rule, the longest intervals encountered during steady repetitive firing (Fig 2C)

against 1/time to peak of late afterdepolarization Time to peak of late afterdepolarization was measured from single spike discharges, generally elicited by antidromic stimulation (Fig 3, inset) and the firing frequencies were measured over half a second of steady repetitive discharge The straight line in the diagram was drawn through points for which the minimum firing frequency (imp/sec) was equal to 1/time to peak of late afterdepolarization (sec [1]) The experimental values follow this line quite closely In the present material no motoneurones displayed steady repetitive discharges at frequencies slower than 1/time to peak of late afterdepolarization (measured from single spike discharges) Fig 8 (line a) shows the regression line calculated from the values of Fig 3 and statistical data are found in Tables I and II

The minimal firing rates were significantly correlated also to 1/time to maximal afterhyperpolarization (Table I) The correlation coefficient for this relation (r = +0 74) was, however, smaller than the one obtained from the values of Fig 3 (r = + 0 98)

Maximal firing frequencies in the primary range

In Fig 4 the maximal discharge frequencies in the primary range, i e within the range of a primary f I slope, were plotted against 1/time to maximal afterhyperpolarization Time to maximal afterhyperpolarization was measured from single spike discharges,

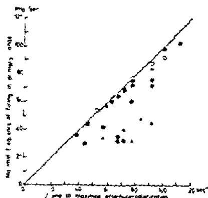


Fig 4

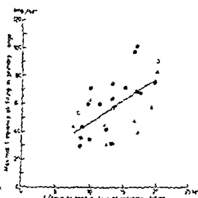


Fig 5

Fig 4 Diagram showing the relation between the maximal discharge frequency within the primary range of firing and the reciprocal value of time to maximal afterhyperpolarization in 32 motoneurones The line is drawn through points for which the maximal frequency of firing within the range of the primary f-I slope = 1/time to maximal afterhyperpola[illegible] cles represent cells able to display repetitive firing at freq[illegible] ing more than 0 5 sec Triangles *represent* cells [illegible] range for as long as 0 5 sec. *Circles represent* unit[illegible] range of primary f-I slopes were obtained only [illegible] repetitive discharges It is *not* known whether *these* units would set u[illegible] sustained firing at frequencies within the secondary range

Fig 5 Diagram showing the relation between the maximal firing frequency within the primary range and the reciprocal value of time to peak of late afterdepolarization Same cells and symbols as in Fig 4 The 16 units whose values were closest to the line in Fig 4 are indicated by wide circles The equation for the regression line is given in Table II

generally elicited by antidromic stimulation The straight line in Fig 4 was drawn through points for which the maximal frequency within the primary range (imp sec) was equal to 1 time to maximal afterhyperpolarization (sec $^{-1}$) The firing rates plotted in Fig 4 are seen to vary between 30—102 imp sec

For each cell in Fig 4 the highest maximal discharge frequency within the primary range was taken irrespective of whether the measurement referred to the initial impulse intervals or to later periods of the discharge This was done because the results of a previous study indicated that the upper limit of the primary range is set by a constant maximal impulse frequency in experiments on adaptation (Kernell 1965 *b* The maximal firing frequencies within the range of a primary f I slope are generally very similar independently of whether they belong to the initial or to the later portion of the discharge (Kernell 1965 *b* and results such as those presented below, but with a somewhat larger spread of the values were obtained also when the measurements were confined to steady firing rates for statistical data, see below

As seen in Fig 4 the maximal discharge frequencies in the primary range for 16 out of 32 cells were within 5 imp sec of 1 time to maximal afterhyperpolarization These 16 motoneurones did not in any other obvious way differ from the 10 cells in which the corresponding discrepancy exceeded 15 imp sec In several of these latter cells firing within the secondary range also clearly occurred at frequencies which were slower than 1/time to maximal afterhyperpolarization However, it should be noted that

much of the spread of values in Fig 4 (*cf* also Fig 5 and Table I) could be due to the errors involved in the measurements The maximal firing rate within the primary range had often to be approximated on the basis of a relatively small number of current steps and the "true" maximal impulse frequency might have required a current strength in between the steps used

In the present material, the intervals obtained from repetitive firing in the primary range were never definitely shorter than the time to maximal afterhyperpolarization obtained from single spike discharges in the same cell (Fig 4) It is in this context of

The same frequencies as in Fig 4 were plotted in Fig 5 against 1/time to peak of late afterdepolarization (see [1]) The values appear to be more scattered in Fig 5 than in Fig 4 This was also the case for the 16 cells whose maximal frequencies in the primary range were most closely correlated with 1/time to maximal afterhyperpolarization

Table II

The relation between the maximum steady firing rate (measured over 0 5 sec) within the primary range and 1/time to maximal afterhyperpolarization had the correlation coefficient r = + 0 53 (28 cells) For the corresponding relation to 1/time to peak of late afterdepolarization the correlation coefficient was r = + 0 52 (28 cells) Both these correlations were statistically significant (t test, $0\,01 > p > 0\,001$)

Absolute maximal firing frequencies of long lasting repetitive discharges

The term "absolute" maximal firing frequency refers to the highest firing rate (imp sec) which could be elicited in a motoneurone, irrespective of whether this maximal discharge frequency occurred in the primary or the secondary range In Fig 6 the absolute maximal firing frequencies of 28 motoneurones were plotted against their times to peak of late afterdepolarization (msec) The latter were measured from single spike discharges generally elicited by antidromic stimulation The cells were stimulated by currents of varying strengths up to a strength which depressed further repetitive firing An approximate measure of the absolute maximal firing rate of long lasting repetitive discharges was obtained by counting the maximal number of spike intervals

between these average values was highly significant (t test, $0\,001 > p$, and there was practically no overlap between the two groups (Fig 6 filled circles and circles respectively) Neurones belonging to these two groups were not significantly different with respect to average values of spike size time to maximal afterhyperpolarization or time to peak of late afterdepolarization *cf* Fig 6) The ability of motoneurones to maintain

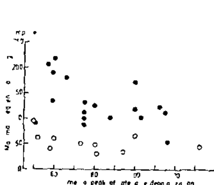


Fig 6

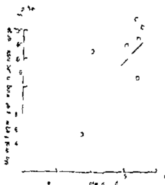


Fig 7

Fig 7 Diagram showing the relation between the maximal discharge frequency in the secondary range of firing and the reciprocal value of time to peak of late afterdepolarization Discharge frequencies were measured over 0 5 sec of constant stimulation The 13 cells included in this diagram were all able to maintain firing in the secondary range during more than 2 sec The equation for the regression line is given in Table II

firing the secondary range is apparently important for making them reach high rates of discharge, as measured over half a second (*cf* Kernell 1965 *b*)

For motoneurones incapable of maintaining firing in the secondary range (Fig 6 circles), the absolute maximal discharge frequencies (measured over half a second), were close to the maximal firing rates in the primary range (*cf* Figs 4 and 5) (Kernell 1965 *b*)

Thirteen of the cells from Fig 6 maintained a discharge in the secondary range during more than 2 sec In Fig 7 the maximal discharge frequencies in the secondary range, i e the absolute maximal firing rates (cf Fig 6) recorded from these 13 cells are plotted against 1/time to peak of late afterdepolarization (sec $^{-1}$) The firing rates of Fig 7 vary between 54—218 imp/sec Much of the spread of values in Fig 7 (*cf* Table I) could be due to similar errors as those involved in the determination of maximal firing rates within the primary range (p 7) The regression line of Fig 7 is shown also in Fig 8 (line c) and the corresponding equation will be found in Table II The maximal discharge frequencies in the secondary range (measured over half a second) were *significantly correlated to both 1 time to peak of late afterdepolarization and 1/time to* maximal afterhyperpolarization (Table I) The latter relation gave a spread of values similar to that shown in Fig 7

The cells of Fig 7 were all capable of firing within the secondary range at frequencies which were steady during 0 5 sec or more At the highest current strengths such steady firing would sometimes be obtained only after a relatively long period of gradual drop in discharge frequency following the onset of current In some of these cells stimulation at the very strongest currents was maintained only during a relatively short time (around 1 sec) and steady firing did not occur during this period of stimulation In several of the 13 cells it could be checked however that the maximal rates of steady discharge

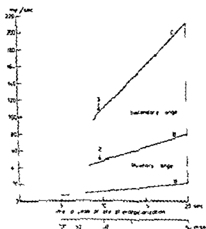


Fig 8 Regression lines showing the relation between limits of discharge frequency and the reciprocal value of time to peak of late afterdepolarization The regression lines were calculated for values earlier shown in Fig 3 (line *a*), Fig 5 (line *b*) and Fig 7 (line *c*) respectively, and the corresponding equations are given in Table II Arrows 1—3 indicate the limits of discharge frequency for cells which have a time to peak of late afterdepolarization of 103 msec Further explanation in text

were of the same order of magnitude as the frequencies plotted in Fig 7 The maximum steady firing rate found was 195 imp/sec (measured over 0 5 sec of constant stimulation)

The discharge frequencies discussed above have all been measured over half a second of constant stimulation However, it should be noted that very high firing rates (up to 300—600 imp/sec) could in most cells be elicited during the initial impulse intervals after the onset of stimulation (*cf* Kernell 1965 *b*)

It does not appear likely that very strong currents which block the soma membrane, would elicit repetitive firing far out in the axon, not recordable by the electrode in the soma During the course of the present work intraspinal motor axons were often penetrated (for criteria, e g Frank and Fuortes 1955), and in these units it was never possible to elicit a steady repetitive discharge by injected current This is consistent with the previous findings that intraspinal motor axons have a fast accommodation when investigated by slowly rising currents In contrast to what has been found for the soma of motoneurones the intraspinal motor fibres always require a minimal current gradient Bradley and Somjen 1961, Sasaki and Otani 1961)

The range of discharge frequencies in motoneurones with different time course of afterpotentials

The main results of the present work are summarized in Fig 8, which shows the regression lines relating the minimal firing frequency (line a, *cf* Fig 3) the maximal firing frequency in the primary range (line b, *cf* Fig 5) and the maximal firing frequency in the secondary range (line c *cf* Fig 7) respectively to 1/time to peak of late afterdepolarization The regression lines are shown for values of time to peak of late afterdepolarization between 50—200 msec which is about the range of variation in duration of afterhyperpolarization found by Eccles *et al* (1958 1961) It should be pointed out that the values represented especially by lines b and c (Fig 8) are to be regarded only as rough approximations (*cf* Table II) and that most observations were made on cells with a time to peak of late afterdepolarization shorter than 120 msec However the diagram gives a general idea about the firing rates occurring in the primary and the secondary range of firing in motoneurones with different duration of afterhyperpolarization the latter measured as was done by Eccles *et al* (1958) It is seen in Fig 8 that motoneurones

TABLE II Equations for regression lines relating limits of discharge frequency to the reciprocal value of time to peak of late afterdepolarization

a Minimal discharge frequency	$= 1.02\,A + 0.8$	$SE_b = \pm 0.03$
b Maximal discharge frequency in primary range	$= 3.21\,A + 15.1$	$SE_b = \pm 0.83$
c Maximal discharge frequency in secondary range	$= 10.33\,A + 4.5$	$SE_b = \pm 3.28$

A — 1/time to peak of late afterdepolarization (sec^{-1})
SE_b — standard error of the regression coefficient (imp/sec/sec^{-1})
Discharge frequencies are expressed in imp/sec
The equations were calculated by the method of least squares from the values shown in Figs 3 5 and 7 respectively

with short afterhyperpolarizations have a larger range of discharge frequencies (*cf* Granit *et al* 1963) The differences between the regression coefficients for lines a and b and for lines b and c respectively (Fig 8, Table II) were both statistically significant (t test $0.05 > p > 0.01$) The data summarized in Fig 8 will be further considered in the Discussion

It should be noted that the limits of discharge frequency were also significantly correlated with time to maximal afterhyperpolarization and time to peak of late afterdepolarization The numerical values of the correlation coefficient for such relations were, however, always a little smaller than the corresponding values shown in Table I

The findings of the present work were also found to be applicable to a selection of cells with high membrane potentials and spike amplitudes

Discussion

The results presented show that in motoneurones stimulated by injected currents the limits of firing rate of long lasting repetitive discharges are significantly correlated with the time course of afterhyperpolarization the latter being measured from single spike discharges (generally antidromic) The time course of the afterpotentials in repetitive firing alters to a considerable degree as a consequence of variations in current strength and discharge frequency (Kernell 1965 *b*) The factors responsible for the time course of the afterhyperpolarization succeeding single action potentials in the non-depolarized state must nevertheless be important in determining the minimal impulse frequency as well as the maximal firing rate within the primary and secondary range respectively An extensive discussion of this point is difficult because relatively little is known about the membrane properties of motoneurones and the complicated mechanisms taking part in the production of a repetitive discharge

The samples in Table I are small and so it is difficult to draw any definite conclusions from the differences between the correlation coefficients Furthermore because of experimental difficulties the measurements of the maximal firing rates within the primary and the secondary range were subjected to an error discussed on p 7 However, some of the findings in Table I raise questions about the possible significance of

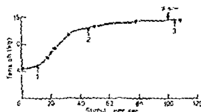


Fig 9 Curve traced from Cooper and Eccles (1930), showing the relation between isometric tension of the medial gastrocnemius muscle and rate of stimulation of the popliteal nerve. Fusion frequency indicated by arrow Arrows 1—3 show to the same frequencies as the corresponding arrows in Fig 8 Further explanation in text

the two measured parameters of the afterhyperpolarization When time to maximal afterhyperpolarization and time to peak of late afterdepolarization were plotted against one another they did, for unknown reasons, to a certain extent vary independently (Fig 1) Not unexpectedly, the minimal firing rate was better correlated with 1/time to peak of late afterdepolarization (*cf* Fig 3) than with 1/time to maximal afterhyperpolarization (Table I) For the maximal firing rate within the primary range the reverse was true (Table I, *cf* Fig 4 and 5) Furthermore, the time to maximal afterhyperpolarization (measured from single spike discharges)seemed to be about equal to the shortest possible spike interval between spikes fired within the primary range (Fig 4) Thus, both the parameters of afterhyperpolarization are of interest for predicting the behaviour of motoneurones in repetitive firing On the basis of these data it is also suggested that the upper and the lower limits of the primary range may, to some extent, be set independently of each other (Table I)

Earlier studies have strongly indicated that the repetitive firing of motoneurones in response to maintained reflex activation is caused chiefly by relatively steady synaptic currents (e g Eccles 1953, p 174 ff, 1964, p 114 ff, Frank and Fuortes 1961, Granit and Renkin 1961) Thus the present results would imply that also under the conditions of maintained reflex activation motoneurones of different durations of afterhyperpolarization would be inherently capable of discharging at different minimal and maximal impulse frequencies as has been earlier suggested by Eccles *et al* (1958) and others It is known from studies of antidromic spike discharges that motoneurones innervating slowly contracting muscles generally possess longer afterpolarizations than those innervating fast muscles (Eccles *et al* 1958, 1961, Kuno 1959) It has also been shown that the motoneurones themselves actually have an influence upon the speed of contraction of the muscles they innervate (Buller, Eccles and Eccles 1960 *a*, *b*) For an effective modulation or reflex tension the contraction speed of the muscle should be matched to the discharge frequencies of its motoneurones (*cf* Adrian and Bronk 1929, Eccles *et al* 1958) It is therefore of interest to compare limits of discharge frequency of lumbosacral motoneurones (*cf* Fig 8) with the results of previously published work on the relation between tension and stimulus rate (Fig 9) in such muscles whose motoneurones have been studied also with respect to the duration of their afterhyperpolarization (Eccles *et al* 1958 1961) The present data were from motoneurones with relatively short afterhyperpolarization (*cf* Fig 6), and the results should therefore in the first instance be compared with the characteristics of fast hind-limb muscles M gastrocnemius medialis will be used for this purpose

The motoneurones of the medial gastrocnemius have an average duration of afterhyperpolarization of 103 msec, counted to peak of late afterdepolarization (Eccles *et al* 1961) According to Fig 8 the minimal discharge frequency for this duration of after-

hyperpolarization would be about 11 imp/sec (arrow 1) and the maximal firing rates within the primary and the secondary range would be roughly 46 imp/sec (arrow 2) and 105 imp/sec (arrows 3) respectively These limits of firing rate predicted from the afterhyperpolarization of medial gastrocnemius motoneurones are indicted by arrows 1—3 in Fig 9, which shows the amount of isometric tension obtained with the medial gastrocnemius muscle at various stimulus frequencies (Cooper and Eccles 1930). It appears (Fig 9) that on the average a medial gastrocnemius motoneurone would not set up a steady repetitive discharge at frequencies slower than those for which the twitches of its muscle start to summate (arrow 1), and that it would not maintain discharges at frequencies much higher than those for which the muscle shows a maximal tetanic tension (arrow 3) The speed of the medial gastrocnemius muscle and the predicted limits of discharge frequency for medial gastrocnemius motoneurones therefore seem to be well fitted to each other

The motoneurones innervating slow muscles (e g soleus) have generally much longer afterhyperpolarization than those studied in the present work The limits of firing rate for motoneurones innervating slow muscles may, however, tentatively be derived from the regression lines of Fig 8 Soleus motoneurones have an average duration of after hyperpolarization of 186 msec (Eccles *et al* 1961) According to Fig 8 their minimal discharge frequency would for this duration of afterhyperpolarization (i e time to peak of late afterdepolarization) be around 6 imp/sec, and their maximal firing rates within the primary and secondary range around 32 imp/sec and 60 imp/sec respectively These predicted limits of firing rate of soleus motoneurones are well in line with the earlier findings that the twitches of the soleus muscle start to summate at a rate of tetanic stimulation around 5/sec, and that the maximal tetanic tension of the muscle is reached at a stimulus rate around 60/sec (*cf* Matthews 1959, Buller and Lewis 1964)

Under the present experimental conditions several motoneurones were unable to fire in a maintained manner in the secondary range, and it is not known whether this was due to damage or to some inherent properties of those cells (Kernell 1965 *b*). Such neurones if naturally occurring, might be unable to maintain the full tetanic tension of their motor units (*cf* Fig 9, arrow 2) It is therefore of interest to note that at least some muscles in the cat (Cooper, Denny Brown and Sherrington 1926) and in man (Bigland and Lippold 1954 Merton 1954) actually may develop full tetanic tension in response to motoneurone activity elicited by synaptic mechanisms

Fig 9 also illustrates the possible physiological significance of firing within the secondary range (*cf* Kernell 1965 *b*) The steeper part of the curve of Fig 9 corresponds to frequencies at which the motoneurones fire in the primary range (between arrows 1 and 2) and the less steep part of the curve falls mainly within a range of frequencies which corresponds to the secondary range of firing of the motoneurones (between arrows 2 and 3) It has been shown that for steady repetitive firing of motoneurones the value of the secondary f I slope is about 2—6 times larger than that of the corresponding primary f I slope (Kernell 1965 *b*) At firing rates in the secondary range, a given increase of excitation to the motoneurones would thus elicit a larger increase in discharge frequency than it would cause in the primary range This is as it were, also in line with the properties of muscle (*cf* Fig 9) because for the final efforts in the production of tension a large increase of firing frequency might be required

Perhaps it should be pointed out that the rough comparisons in the previous para graphs were made only for the sake of discussing some general principles Of course further experimental work would be required for a definite answer to these and other

problems concerning the relation between the repetitive firing of individual motoneurones and the tension developed by their motor units under various circumstances.

The minimal frequencies of steady discharge previously obtained from cat lumbosacral motoneurones in response to reflex stimulation have been around 5—20 imp sec (e g Denny Brown 1929 *a* Granit, Haase and Rutledge 1960) As the duration of afterhyper polarization is known to be between about 50—200 msec (Eccles et al. 1958 1961) these data are well in line with the present results (*cf* Fig 8 line *a*) The maximal impulse frequencies obtained by injected currents in the long lasting discharges of the present work were often considerably faster than those previously found with reflex stimulation (*cf* Adrian and Bronk 1929 Denny Brown 1929 Granit *et al* 1960) The fastest firing rates of the maintained discharges of reflexly activated cat lumbosacral motoneurones have been around 60—90 imp/sec (quadriceps motoneurones Adrian and Bronk 1929) However, there is no definite indication in the earlier investigations that the maximal firing rates of reflexly actived motoneurones were determined by the inherent properties of the motoneurones themselves For a more detailed comparison of the present findings with repetitive reflex firing the duration of afterhyperpolarization has to be known for the individual motoneurones investigated

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Spectral Sensitivity of Single Units in the Cortical Area Corresponding to Central Vision in the Monkey[1]

By

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Abstract

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Physiological evidence bearing on the Young-Helmholtz and Hering theories of colour vision has been slow to accumulate. Marks (1963) demonstrated that single cones contained one or the other three pigments substantiating Young's basic postulate. Early microelectrode studies in the retina demonstrated, too, that the responses of single units after strong monochromatic illumination fell into three groups maximally sensitive to blue, green and red (Granit 1945). Later studies demonstrated opponent colour responses in the retina: elements peripheral to the ganglion cells responded oppositely to opponent colours and retinal ganglion cells could be brought to do so when center or the periphery of their receptive fields was stimulated (summarized by MacNichol 1964). This opponent pattern of response is transmitted to the geniculate in the monkey, where single units responded oppositely, that is in an on or an off fashion, to opponent colours green-red, though the flashes illuminated the retina diffusely (De Valois 1960, De Valois, Jacobs and Jones 1962). Other units, on-cells, responded over the whole spectrum but with well defined peaks of sensitivity (De Valois 1960).

[1] This investigation was supported in part by Public Health Service Grant NB-03943-02 from the Division of Research Grants.

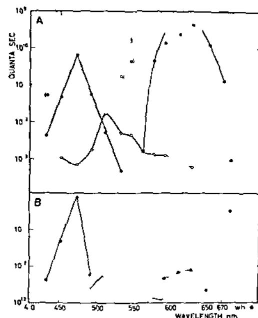


Fig 1 Combined spectral sensitivity curves of units in the cortical area corresponding to central vision in the monkey
A Low criterion of threshold responses to at least about a fifth of the presentations The arrows indicate responses at the lowest available intensities The ( ) indicate criterion responses at lower but not at intermediate intensities
B High criterion of threshold the highest reliable number of responses to at least a third to a half of the presentations

		Sensitivity		
		blue	green	red
Number of units		8	8	8
Number of presentations		17	23	23
Criterion number of responses	A	4	5	5
	B	7	7	11

The single symbols on the right indicate the two responses to white with the criterion used

In the cortex of the monkey in an area corresponding to 15° from the fovea single units responded essentially as in the geniculate either as on responses with broad spectral sensitivity or in an on-off fashion oppositely to opponent colours (Motokawa Taira and Okuda 1962) In the cortical area of monkey corresponding to the fovea or 1° to 2° displacement from it we did not find reproducible opponent colour responses in single units those single units which responded reproducibly to restricted portions of the spectrum did so to blue green or red (Andersen Buchmann and Lennox Buchthal 1962)

The method of determining the spectral sensitivity of these single units has been described (Andersen *et al* 1962) Briefly the monkeys were lightly anesthetized with chloral se urethane and 8 msec flashes were delivered once a second to one eye in Maxwellian view single units

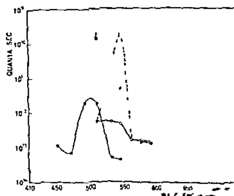


Fig 2 The spectral sensitivity of the

○ Four units (10 presentations) responding to green Criterion of threshold, four responses The interrupted line indicates the uncertain thresholds
( ) Criterion responses at lower but not at intermediate intensities

appeared only twice with the criteria used (Fig 1) The 3 single units responding … were not included, since their threshold was not determined

The blue sensitive units showed a clear peak in spectral sensitivity at 475 … responded up to 530 nm with the low criterion of threshold (Fig 1A) There … few responses to 410 …

criteria (not shown in Fig 1B)

The green-sensitive units did not respond …

rupted curve, Fig 2), and ii) responses oftenest to blue-green and … that is from 450 nm to 545 nm with scattered responses above … peaks of sensitivity at about 500 and 590 nm and with few responses … line Fig 2) The responses in this latter group were especially … green-sensitive units according to their predominant kind of … in Fig 2 were obtained However, there was no clear division … in this group, since any one unit could respond to green at one … and yellow during another trial or during another portion of …

Thus, the organization of units in the cortex corresponding to central vision is essentially trichromatic with clearly defined blue- and red-sensitive units and with green sensitive units which responded sparsely, variably, and which could respond in a vaguely opponent colour fashion to about 500 and 590 nm How is this trichromatic code derived from the opponent colour code in the geniculate (De Valois 1960 De Valois Jacobs and Jones 1962) Our working hypothesis is that the "on" characteristics of each on-off" geniculate cell are found in the colour-coded cortical units In subsequent work (unpublished observations with C Guld) we have used long as well as short flashes and have not found true off" responses among the clearly colour-coded cortical units The latency of the unit's responses may be long, but prolonging the flash demonstrates that they are not true off" responses We assume, then, that the extreme red sensitivity of the red on' cells in the geniculate (De Valois et al 1962) corresponds to the red-sensitive units in the cortex, the extreme blue sensitivity of the "green-on" units in the geniculate (De Valois et al 1962), to the blue-sensitive units in the cortex The green sensitive units in the cortex must, we think, reflect the "on" sensitivity in the middle range of the spectrum of both "red-on" and green-on" units in the geniculate (De Valois et al 1962) Work is under way to test this hypothesis

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Analysis of the Efflux Kinetics of Sodium from Small Cylinders of Rabbit Kidney Cortex

By

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Abstract

Bojesen, E., P. P. Leyssac and B. S. Nielsen. *Analysis of the efflux kinetics of sodium from small cylinders of rabbit kidney cortex.* Acta physiol. scand. 1965. *65.* 105—119. — Based upon two different two-compartment models, a non-communicating and a more realistic communicating two-compartment system, the efflux curves from two cortical cylinders of rabbit kidney were subjected to a graphical and a numerical analysis respectively. A fairly good correspondance was obtained between the results of both types of analysis and a surprisingly good fit with the experimental data was obtained by the numerical analysis. The results of the latter analysis showed that in the cylinders 85 % and 80 % of the exchangeable sodium was in diffusion equilibrium with the medium. The diffusion coefficient was calculated to be 7.3×10^{-6} cm²/sec at 25° C and 13×10^{-6} cm²/sec at 40° C. From separate determinations of the average concentration of exchangeable sodium in tissue water in a serie of cylinders of similar dimensions as those used for the analysis of the efflux curves it was calculated that the fraction of exchangeable sodium which is not in diffusion equilibrium with the medium (15—20 %) occupied a fraction of the tissue water of 30—45 % at a sodium concentration of about 55—40 meq/l. Taking an area to volume ratio corresponding to a single proximal tubule, a flux rate was calculated and found to be of an order of 10—100 times greater than that obtained in striated muscles by different investigators.

In a previous paper (Bojesen and Leyssac 1965a) data were presented which raised the question whether an active transport of sodium responsible for the sodium concentration gradient between the medium and the tissue water, known to be dependent upon the metabolism of the tissue (Mudge 1951, Whittam and Davies 1953), might at all be demonstrable by kinetic studies in the case of a large sodium pool in diffusion equilibrium with the medium. Of most significance for this doubt was a close equality in volumes of distribution and efflux rates observed in experiments with ^{35}S-labelled sulphate (at a concentration of 6—10 mM) and ^{22}sodium. Taking into account the difficulties offered by the complicated kinetics of the diffusion process in slices of all

defined thickness to an analysis of the temperature dependency of the process, it was furthermore impossible to demonstrate on this basis any significant deviation from the diffusion kinetics, in opposition to the conclusion drawn by Lassen and Thaysen (1961) This negative result was roughly in agreement with the result of Mudge (1953) and other data on the rate of uptake of sodium by cortical slices (Davies 1954, Whittam and Willis 1963) It remained, however, possible that a quantitative analysis based on an improved technique might allow a determination of the diffusion coefficient of sodium in kidney cortical tissue in vitro and the detection of a minor rapidly exchangeable intracellular sodium pool

Thin slices of kidney cortical tissue are unsuitable for this purpose for several reasons First of all it is impossible to determine the distribution of a substance on the different thickness of the slice, a parameter, which is essential for the determination of a diffusion coefficient, since the thickness in the second power determines the rate of diffusion Secondly, the zero value of the activity in the tissue will always remain ill defined no matter how one proceeds, because of the relatively very large and ragged surface area Incubation medium adhering to this surface will be rinsed off in the very beginning of the wash out process and will therefore invariably cause an ill defined overestimate of the zero value Thirdly, some fractions of a thin slice may be so thin that washing medium actually might flow through holes or intertubular spaces, rendering calculations according to an average thickness meaningless

Optimal conditions for a quantitative analysis suitable for the detection of a rapid exchange process for sodium would be such in which a maximum rate of sodium loss by diffusion and an adequate oxygen supply is combined with well-defined geometry and sturdiness of the isolated piece of tissue Cylinders of kidney cortex fulfill the requirement of well-defined geometry, but it turned out in practice to be difficult to obtain sturdy smooth and regular cylinders of some length with diameters less than 1 1—1 2 mm This technical difficulty raised the question whether an adequate oxygen supply by diffusion was at all obtainable with well defined geometry, since it is generally accepted that slices of liver and kidney tissue must have a thickness less than 0 3—0 5 mm if the central parts shall be sufficiently oxygenated by diffusion at an oxygen tension of 1 atmosphere in the medium (Umbreit *et al* 1957) With an oxygen consumption in renal cortical tissue of 0 5—2 0 micromoles/g tissue/min at 37° C (Lassen and Thaysen 1961 Whittam and Willis 1963) and a Q_{O_2} of about 2 the oxygen requirement does not exceed the supply obtained by diffusion from the entire tissue of cylindrical geometry at 25° C and for about 80 % of the total mass at 37° C (diameters of 1 1—1 2 mm and an oxygen tension of 1 Atm in the medium) when calculated according to the equations 25 31 and Fig 3 given by Hill (1929) It was therefore considered to be feasible although at the borderline of safety to obtain a well-defined geometry and at the same time adequate oxygen supply by diffusion

Methods

Rabbits were killed by neck stroke and the kidneys removed immediately Cortical cylinders ... were ... ation ... meg ... Atm. ... ning

Results and calculations

In order to obtain a correct value of the activity remaining in the cylinder at any time, the ratemeter curve must be corrected for 1) the zero setting of the ratemeter, 2) the background activity of the counter, and 3) the activity in the chamber outside the cylinder. This last correction, a "dead space error", is evaluated as follows

The total activity remaining in the chamber at any time t^o, $S(t^o)$, is the sum of the activity in the cylinder, $Q(t^o)$, and the activity in the one ml volume of the flushing medium, $B(t^o)$. From the assumption of a perfect mixing in the chamber therefore

$$\text{(a)} \quad S(t^o) = Q(t^o) + B(t^o) \cdot 1$$

Since the flow rate was 16 ml per min, we have

$$\text{(b)} \quad -\frac{dS(t)}{dt} = B(t) \cdot 16$$

Combining (a) with (b) we get

$$\text{(c)} \quad Q(t^o) = S(t^o)\left(1 + \frac{\frac{dS(t^o)}{dt}}{16\, S(t^o)}\right)$$

From equation (c) the activity in the tissue at any time, t^o, is seen to be equal to the observed total activity times one plus the relative rate of change of total activity in 1/16 of a min at this particular time. This correction was only significant for the initial 2 min and did not exceed 5 per cent at 25° C and 9 per cent at 40° C for the values used in the analysis

A. *Graphical analysis of the corrected ratemeter curve*

Diffusion kinetics of a substance dissolved in a homogeneous body of cylindrical geometry, the length of which is large compared with the radius, can be described the equation

$$\text{(1)} \quad \frac{Q(t)}{Q(o)} = 4\left(\frac{e^{-\frac{D v_1^2 t}{a^2}}}{v_1^2} + \frac{e^{-\frac{D v_2^2 t}{a^2}}}{v_2^2} + \frac{e^{-\frac{D v_3^2 t}{a^2}}}{v_3^2} - \right)$$

This equation has been derived from the general law of diffusion by for instance Hill (1929) and Jacobs (1935). It says that the amount of substance remaining in the

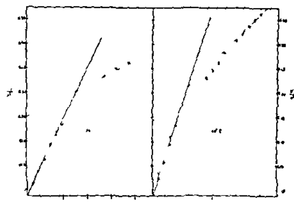


Fig 1 A plot of the function $\left(\frac{Dt}{a^2}\right)$ of $\frac{Q(t)}{Q(o)}$ versus time (min recorded from two cylinders at two different temperatures

homogenous cylinder with a radius (a) at a time (t), (Q(t)), is equal to 4 times a series of exponential terms, in which the time constants are products of a common factor (D/a^2) (the diffusion coefficient over the radius of the cylinder in the second power) and a series of numbers (t_1 t_2 t_3 etc) which are the zero values of the Bessels function $J_0(x)$ with the numerical values 2 405 5 520, 8 654 etc From this equation a table between corresponding values of (Dt/a^2) and Q(t)/Q(o) has been calculated by Hill (1929, Table IV) Since any observed value of Q(t)/Q(o) at an observed (t) will correspond to a value of (Dt/a^2), given in the Table (Hill 1929) D/a^2 may be determined by graphical analysis This procedure is analogous to the well known graphical analysis of a logarithmic function Thus if the process studied does follow the kinetics of diffusion in a cylinder, such a plot will of course yield a straight line through zero with a slope equal to D/a^2

Fig 1 shows such a plot for two different cylinders of renal cortical tissue flushed at 25° and 40 C It clearly demonstrates that the efflux kinetics of sodium does not follow the expected straight line characterizing diffusion However a clear deviation from the diffusion kinetics is not observed in the first two min in which about 70 per cent of the initial activity is lost Thus an analysis according to a two component system seems appropriate

B *Graphical analysis of the efflux curve in terms of a non communicating two compartment model*

Keynes (1954 has discussed the possibility of performing a graphical analysis of the efflux kinetics of sodium in striated muscles of cylindrical geometry assuming sodium to be distributed in two communicating compartments (extracellular fluid volume and the intracellular volume) An explicit expression suitable for graphical analysis was however not obtained and a mathematical analysis of the proper model was used for evaluation of the error introduced by the graphical analysis according to a simplified model The simplified model was one in which the two compartments were considered to be non-communicating In case of small striated muscles this error was shown to be of some although not of serious importance because of a great difference between the rates of loss from the two compartments In the present case Fig 1 shows directly that we cannot expect a similar favorable situation when kidney cortical cylinders are used

Particularly the evaluation of the slow component must become greatly erroneous and the rate constant seriously underestimated. The graphical analysis is, however, of value as a reference for the numerical analysis of the data according to the proper model.

$$F = \frac{C_u E}{C} = \frac{E}{N} \qquad (2)$$

N may be determined by direct analysis of the incubated cylinders and was found to be 0.7—0.8 at 25° C when C_u was 140 meqv. per liter, as in the present experiments (Table I). By these terms the following relationship exists between the sodium concentration of the two compartments

$$\frac{C_v}{C_u} = \frac{(N-E)}{(1-E)} \qquad (3)$$

If the concentration of activity in the E-compartment is denoted by (u) and that in the (1—E) compartment by v, the following equation describes by definition the exchange process of sodium between the two compartment (Keynes 1954)

$$\frac{dv}{dt} = \frac{M\,A}{V}\left(\frac{u}{C_u} - \frac{v}{C_v}\right) = K\left(u - v\,\frac{(1-E)}{(N-E)}\right), \qquad (4)$$

in which (M) means the flux rate (meqv/cm² sec) and (A) and (V) means area and volume of the structural unit representing the (1 — E) compartment. The term (K) is merely a common constant comprising some characteristics of the system

$$K = \frac{M\,A}{V\,C_u}$$

In the case the system is considered to consist of two non-communicating compartments (u) will be zero for all values of (t). Solving equation (4) we then get

$$\frac{v(t)}{v(o)} = e^{-tK\frac{(1-E)}{(N-E)}} \qquad (5)$$

Since the loss of activity from (E) is described by the equation for diffusion in a cylinder (eq. 1) the following equation describes the two integrated processes in terms of the simplified model

$$\frac{Q(t)}{Q(o)} = F\,4\left(\frac{e^{-\frac{D r_1^2 t}{a^2}}}{z_1^2} + \frac{e^{-\frac{D r^2 t}{a^2}}}{\sigma_2^2} - \quad\right) + (1-F)\,e^{-tK\frac{(1-E)}{(N-E)}} \qquad (6)$$

In Fig. 2 the log of Q(t)/Q(o) is plotted against time. As soon as the first term of equation (6) becomes negligeable relative to the second term the curve will be described by this latter term with a slope equal to $K\frac{(1-E)}{(N-E)}$. An extrapolation of this part of the curve will intercept with the ordinate at a value of log (1 — F). The results obtained at two different temperatures from each of the two cylinders are given in Table II. It is obvious from the Fig. 2, that an analysis according to the extrapolation is a rough approximation only, stressing the necessety of a numerical analysis.

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Fig.
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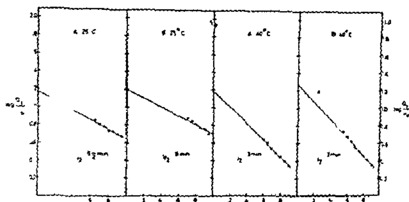


Fig 2 A semi logarithmic plot of recorded $\frac{Q(t)}{Q(o)}$ versus time (min) from the two cylinders at 25° and 40° C. The intercept with the ordinate of the tangent to the final slope indicates the value of the non-diffusible fraction of sodium $(1 - F)$

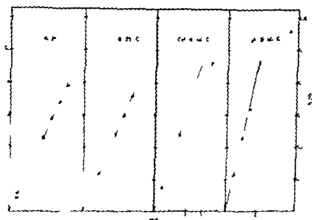


Fig 3 A plot of the function $\left(\frac{D\,t}{a^2}\right)$ of $\frac{Q(t)}{Q(o)}$ versus time (min) recorded from the two cylinders at 25 and 40 C ● integrated diffusion and exchange processes ▲ after subtraction of the exchange process

C *Numerical analysis of the efflux curve in terms of a communicating two-compartment model*

The total activity present in the cylinder at a time (t) is described by the equation

$$Q(t) = \int_0^a 2\pi r L f(r\ t)\, dr \quad \text{where} \quad f(r\ t) = (1-E)\,v + E\,u.$$

In terms of the communicating two-compartment model this function has two properties which can be described by partial differential equations (Keynes 1954)

(7) $$E\,\frac{\partial u}{\partial t} = E\,D\left(\frac{\partial^2 u}{\partial r^2} \quad \frac{1}{r}\,\frac{\partial u}{\partial r}\right) - (1-E)\,\frac{\partial v}{\partial t}$$

This equation simply says that the amount of activity lost from the fraction E of a differential volume of the cylinder at a distance (r) from the center in the time interval (dt) is the amount

lost by diffusion minus the amount added from the $(1-E)$ fraction of the same differential volume in the same time interval (dt)

(8) $$\frac{\delta v}{\delta t} = K(u - \frac{1-E}{N-E}v)$$

Equation (8) expresses as in equation (4) that the rate of loss of activity from the $(1-E)$ compartment in the distance (r) from the center of the cylinder is proportional to the difference between the specific activities of sodium on the two s des of the boundary between the two compartments

The boundary conditions are $u = y \quad o$ for $r \quad a$ and $o < t$ $u(r\ o)$ and $v(r\ o)$ are con stants u_o and v_o having the ratio

$$\frac{u_o}{v_o} = \frac{1\ \ E}{N\ \ E} = c.$$

The known constants of the problem are a N and we want to find the values of E D and K giving the closest fit to the observed function Q (t)

We assume a solution

$$u \quad R\,U \text{ and } v \quad R\,V$$

where R is a function of r alone and U and V are functions of t alone By the theoreme of uni queness we know that if such a solution exists it will be the solution sought for

Putting the express ons for u and v into (7) we obtain

$$(1-E)R\,V + E\,R\,U = E\,D\ (U\,R \ + U\,R/r)$$

where primes denote derivation with respect to the independent variable in question

The equation may now be separated in

(9) $$R \ + R/r + \frac{k^2}{ED}R - 0 \text{ and}$$

(10) $$(1-E)V \ + E\,U \ + k^2U = 0$$

where k^2 is the constant of separation

The general solution of (9) is

$$R = A\,J_0\,(\frac{k}{\sqrt{ED}}r) + B\,Y_0\,(\frac{k}{\sqrt{ED}}r)$$

where A and B are arbitrary constants The second term may however be discarded as our solution has to rema n finite for $r - 0$ Subst tution of $u \quad RU$ and $v = RV$ in (8) yields

(11) $$V = K(U - cV)$$

We may now eliminate U between (10) and (11) and have

(12) $$(E/K)V \ + (1-E+Ec+k^2/K)V \ + k^2cV = 0$$

Here the general solution is

(13) $$V = ae^{\mu t} + be^{\nu t}$$

where μ and ν are the solutions of the quadratic

(14) $$z^2E/K + (1 \ \ E + Ec + k^2/K) \ + k^2c = 0$$

and a and b are arbitrary constants

The solution is valid only when $\mu \neq \nu$ which will prove to be the case

The general solution for v now is

$$v \quad \Sigma\,J_0(\frac{k}{\sqrt{ED}}r\ (ae^{\mu t} + be^{\nu t})$$

for which the general solution for u follows when put into (8)

$$u - \Sigma\,J_0(\frac{k}{\sqrt{ED}}r)\ ((\mu/K + c)\,ae^{\mu t} + (\nu/K + c)\,be^{\nu t})$$

The boundary conditions for v (and u) demand that we chose for k values the numbers

$$k_i \quad v_i\frac{\sqrt{ED}}{a}$$

where v_i are the solution of $J_0(v) - 0$ The μ and ν values are the corresponding solutions of (14)

To determine the corresponding values of a and b say a and b we put $t = 0$ and get

$$v_0 = \sum_{=1}^{\infty} J_0(v_i \; r/a)\,(a + b) \quad \text{and}$$

$$u_0 = \sum_{i=1}^{\infty} J_0(v_i \; r/a)\,((\mu/K + c)a + (\nu/K + c)b)$$

The general theory for the development of a function in terms of Bessel functions gives

$$a + b = \frac{2v_0}{a^2(J_0(v))^2}\int_0^a r\,J_0(v_i \; r/a)\,dr$$

or

(15) $$a + b = A$$

where $$A = \frac{2}{v\;J_1(v)}$$

From the expression for u_0 we get in the same way

(16) $$(\mu\;K + c)a + (\nu\;K + c)b = A\;u_0$$

Solution of (15) and (16) with respect to a and b gives

$$a = A\,\frac{\nu}{\nu - \mu}\;v_0$$

and

$$b = A\,\frac{\mu}{\mu - \nu}\;v_0$$

In what follows we put $\nu - \mu = q$

Now

$$f(r\;t) \quad v_0 \sum_{=1}^{\infty} A\;J_0(v\;r/a)\,h\;(t)$$

where

$$h\;t \quad 1 - E - Ec + E\mu\;K\;e^{\mu}\;\nu/q - (1 - E + Ec + E\nu/K)\,e^{\;t}\,\mu/q$$

For $Q\;t$ we get

$$Q(t) = 2\pi L\,v_0 \sum_{=1}^{\infty} A\;h\;(t)\int_0^a r\,J_0(v\;r/a)\,dr$$

$$4\pi L\,v_0\,a^2 \sum_{=1}^{\infty} \frac{h\;(t)}{v^2}$$

If we put $1 \quad E \quad cE = Eg$ and use $\mu\;\nu = \dfrac{K\,k^2\,c}{E} = v^2\;\dfrac{KD}{a^2}$ we may write

$$Q\;t \quad 4\pi L\,v_0\,a^2\,E \sum_{1}^{\infty}\left[\left(\frac{g\nu}{v^2} - \frac{D\,c}{a^2}\right)\frac{e^{\mu}}{q} \quad \left(\frac{g\mu}{v^2} + \frac{D\,c}{a^2}\right)\frac{e^{\nu}}{q}\right]$$

Denoting the factor in front of the Σ by C and the coefficients of the exponentials by m and n we get

(17) $$Q\;t \quad C \sum_{1}^{\infty} (m_i e^{\mu\,t} \quad n\;e^{\nu})$$

$$\sum \quad M\,e^{\mu} \quad N\,e^{\nu}$$

The actual calculation was done in the following way

Equidistant values of the $Q\;t$ function $Q_0\;t_j$ and $j = 0\;1\;2 \ldots p$ were obtained from the originally recorded trace by drawing an even curve through the trace and applying the corrections mentioned above p 3–4. The obtained values are denoted Q_0. Preliminary values of E, D, K from the graphical analysis of the same data in terms of the simplified model were given to the computer the GIER of the Mathematical Institute of the University of Copenhagen

TABLE I From the wet weight of the cylinders and the sodium concentration of the total tissue, *the average concentration of exchangeable sodium in total tissue water (C) was* calculated taking total water space as 0 8 of wet weight (Robinson 1950, Whittam and Davies 1953) The sodium concentration of the incubation medium (equal to C_0) was *140 meqv/l* H_2O *N is calculated from* $\frac{C}{C_0}$

Tmp	No of cyl	Wet weight mg	Exchang Na meq/kg wet w	Exchang Na meq/kg H_2O	N
		av range			
25°C	6	10 5 (8 5—12 5)	81±7 (s d)	100±9 (s d)	0 7 0 8
38 C	8	9 (7—11)	88±4	110±5	

In the computer the corresponding values of m_i μ_i n and ν were calculated and the constant C computed to minimize

$$S = \sum_{j=0}^{j=j_{max}} (Qo(t_j) - Qc(t_j))^2 / P^2_j$$

[illegible]

minimize S[1]

The resulting values of $M_i = C\,m_i$, $\Lambda_i = C\,n_i$ and μ_i and ν_i are used for the calculation of discrete values of $Qc(t_j)$ which were redressed by the previously applied corrections to correspond to the original trace These values were plotted in the traces and shown in Fig 4 It is seen that it will be very difficult to draw a smooth curve, which will fit the recorded trace better than one through the calculated values, and for all values were the differences between the smooth curve drawn a priori and the calculated curve smaller than P_j From the discontinuity of the boundary conditions as t = o, as an expression of the Gibbs phenomenon, a systematic distribution of the small differences between the a priori values and the calculated values *was to be* expected and actually observed

The direct search is a safe but very slow method A linearization method would be much faster, but the numerical delicacy of the problem would probably cause the computation *to diverge in many cases With 20 exponential terms and 20* values of (t) the calculation time was about 20 min The sensitivity of the result of the numerical analysis to preliminary values of E, D and K *from the graphical analysis was checked* by giving the computer values differing by 30 % in the opposite direction of the observed difference between the two results This procedure was found to be of no significance to the result of the numerical analysis It can therefore be concluded that the numerical analysis offers a well-defined description of the data in terms of the proposed model

[1] A copy of the ALGOL-programme will be sent by the authors on request

TABLE II Data from graphical and numerical analysis of efflux curves from two cylinders at tissue sodium in diffusion equilibrium with the medium. F the fraction by volume sodium concentration in tissue water over sodium concentration in the medium $F = \frac{MA}{V C_0}$ (see p 5)

	Cyl radius (cm)	Diff coeff Na D' 10⁶ cm² sec⁻¹ 25°	40°	F 25°	40°
Non-communicat. two-compart. model	0 060	6 5	9 1	0 85	0 84
	0 055	6 5	11 0	0 85	0 82
Communicat. two-compart model	0 060	7 3	10 4	0 87	0 84
	0 055	7 3	15 3	0 83	0 77

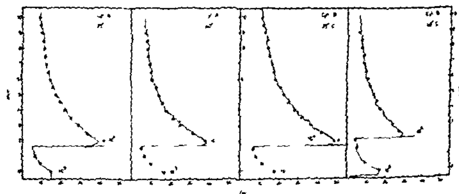


Fig 4 Superimposed on the directly recorded trace of Q (t) during the efflux (continuous curve) are plotted corresponding equidistant calculated values computed from the communicating two-compartment model

Discussion

A basic condition which must be fulfilled before one can accept the model underlying the calculations is that the calculated diffusion coefficient of sodium comes out with a reasonable magnitude and temperature dependency The free diffusion coefficient of sodium in water at 25 C is, according to calculations by several authors see Harris and Burn 1949 Keynes 1954 Johnson 1955 about 12 × 10⁻⁶ cm² sec⁻¹ The virtual diffusion coefficient in the present experiments was found to be about 7 3 × 10⁻⁶

two temperatures D virtuel diffusion coefficient of sodium in tissue F fraction of total of tissue water in which sodium is in diffusion equilibrium with the medium N the average $\left(\frac{C}{C_m}\right)$ k time constant of the exchange process of sodium from the (1 — E) space

N 0.8				N 0.7			
Calc space for free diff E=FN		Rate constant of Na exchange $10^3k = k\frac{1-E}{N-E}$ 10^3 sec^{-1}		Calc space for free diff E=FN		Rate constant of Na exchange $10^3k = k\frac{1-E}{N-E}$ 10^3 sec^{-1}	
25°	40°	25°	40°	25°	40°	25°	40°
0.69	0.67	1.9	3.7	0.60	0.59	1.9	3.9
0.68	0.65	2.1	3.9	0.60	0.57	2.1	3.9
0.70	*0.67*	*2.0*	*4.8*	*0.61*	*0.59*	2.3	5.6
0.66	0.62	2.8	6.0	0.58	0.54	3.0	5.5

at 25° C. In the case of muscles this reduction is readily explained, according to Harris and Burn (1949), on the basis of the heterogeneity of the tissue system and can be considered as an effect of a reduction of the differential area available for diffusion On this background one may understand that the virtual diffusion coefficient of sodium in kidney cortical slices is higher than in striated muscles, since the relative volume of the heterogeneous system available for diffusion is also found to be greater in kidney cortical tissue The temperature coefficient (Q_{10}) may be calculated as a measure of the temperature dependency of this virtual diffusion coefficient, and was in the present two experiments 1.3 and 1.7 In the evaluation of these figures one must have in mind that each value is based upon data of two successive experiments with the same cylinder, that the tissue is fairly elastic, and that the calculated diffusion coefficient is a function of the radius in the second power These experimental circumstances imply that a determination of a true Q_{10} must be based upon a series of experiments The two values given should consequently be considered as examples only One may conclude that with respect to magnitude and temperature dependency the obtained diffusion coefficients of sodium supports the validity of the model The present finding that a 15—20 % fraction of the exchangeable sodium pool is not in diffusion equilibrium with the medium may apparently be difficult to reconcile with the previously reported identity (within 5—10 %) of calculated distribution volumes of sodium and sulphate (6—10 mM) in slices (Bojesen and Leyssac 1965) It is, however, most likely that this approximative identity is not real but is due to sulphate accumulation in subcellular particles of partially lyzed cells since Davies *et al* (1961) have reported sulphate accumula-

TABLE II Data from graphical and numerical analysis of efflux curves from two cylinders at tissue sodium in diffusion equilibrium with the medium F the fraction by volume sodium concentration in tissue water over sodium concentration in the medium

$K = \frac{MA}{\sqrt{C_u}}$ (see p 5)

	Cyl radius (cm)	Diff coeff Na D 10^6 $cm^2 sec^{-1}$ 25°	40°	F 25	40
Non communicat two compart model	0 060	6 5	9 1	0 85	0 84
	0 055	6 5	11 0	0 85	0 82
Commun cat two-compart model	0 060	7 3	10 4	0 87	0 84
	0 055	7 3	15 3	0 83	0 77

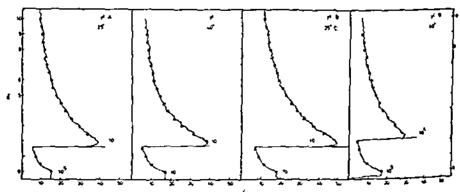


Fig 4 Superimposed on the directly recorded trace of Q(t) during the efflux (continuous curve) are plotted corresponding equidistant calculated values computed from the communicating two-compartment model

Discussion

A basic condition which must be fulfilled before one can accept the model underlying the calculations is that the calculated diffusion coefficient of sodium comes out with a reasonable magnitude and temperature dependency The free diffusion coefficient of sodium in water at 25 C is according to calculations by several authors (see Harris and Burn 1949 Keynes 1954 Johnson 1955) about 12 × 10⁻⁶ cm² sec⁻¹ The virtual diffusion coefficient in the present experiments was found to be about 7 3 × 10⁻⁶

is, however, roughly in agreement with the difference of 10—30 mV in intracellular electrical potentials of kidney cortical cells in situ as opposed to slices reported by Whittembury (1964) Kidney cortical tissue under the present and mostly used condi- [illegible]

equilibrium with the medium with respect to sodium It is of course possible that this variation corresponds to characteristic structural segments of the nephrons. If this is so most of the resistent fraction must include certain parts of the proximal tubules since the (1-E) compartment is 2—3 times greater than the fraction of distal tubules in the cortex

The Q_{10} of the exchange process of sodium between the two compartments was found to be about 1 7, which is equal to that found for striated muscles and the frog skin according to Ussing (1960, Table V) This value is independent of the chosen value of N, but rely of course on the assumption that N has been the same under both experimental conditions (25° and 40° C)

The fairly high Q_{10} and the fact that incubated cortical tissue in vitro maintains a sodium concentration lower than that of the medium invites to a calculation of the flux rate $\left(M = \frac{K\,V\,C_n}{A}\right)$ which amounts to 240—350 peq/cm² sec (N = 0 7 and 0 8 respectively) taking the ratio V A = r/2 and r as the outer radius of the proximal tubules in vitro (r = 20 μ) (Bojesen and Leyssac 1965) This flux rate may be compared with the sodium flux of striated muscles, which on an average was found to be 5—10 peq/cm² sec at about 20° C by a number of investigators (see Keynes 1954), and with the net flux rate in proximal tubules in vivo of about 3000 peq/cm² sec found by Windhager and Giebisch (1961) The latter figure corresponds closely to an occlusion time of 20 sec measured by Leyssac (1963) at relatively low filtration rates, using the observed radius of 12—13 μ in the calculation The flux rate of the best preserved fraction of the incubated tissue is therefore of the order of 10 times less than average rate of net transcellular transport of the proximal tubules in the filtering kidney in vivo A comparison between the in vitro recorded flux rate of sodium and net transfer rate under physiological in vivo conditions seems however only meaningful if the in vitro flux rate may be considered to be a transcellular transport in contrast to a process analogous to that of sodium extrusion from other types of tissue (e g muscle, nerve, etc), in which it only serves the maintenance of the intracellular electrolyte composition It is not quite impossible that ions may gain access to the brush border (luminal) side of still functioning cells by diffusion through disintegrated neighbouring or opposed cells of the same tubule If, further, it is assumed that transcellularly transported sodium is mixed with the intracellular (cytoplasmatic) sodium pool and that sodium is actively extruded exclusively at the basal (peritubular) side of the proximal cells, then the flux rate measured in cylinders in vitro will actually represent a true transcellular transport rate Only in the case that all these assumptions are accepted will it be reasonable to relate the measured in vitro flux rate to the 10 times greater net flux rate in the functioning kidney and to the suprabasal oxygen consumptions of cortical tissue in these two states (in vitro versus in vivo at an average rate of reabsorption) It then turns out that the flux rate as well as

the oxygen consumption in vitro is reduced by approximately the same factor (assuming in agreement with the recent observation by Blond and Whittam (1964), that preserved and lysed cells are about equally responsible for the sodium dependent oxygen consumption in cortical tissue in vitro of 0 5—1 0 μmole O_2/g tissue/min observed by Lassen and Thaysen (1961) and by Whittam and Willis (1963)) Any attempt to approximate the in vitro tissue function to the in vivo state of the cells by improvement of the medium until maximum oxygen consumption must be based upon all these assumptions It does, however, appear more likely that the flux rate measured in vitro has nothing to do with the normal transcellular transport process, but rather is a measure of the process responsible for the maintenance of the intracellular electrolyte composition If this be true, it is a measure of the steady state flux at a balance point of passive sodium permeability and the sodium stimulated extrusion process In this case the alternative of whether a tubule cell will become lysed or not will depend upon whether the balance point can be reached at an intracellular sodium concentration low enough to ascertain a sufficiently small cell volume and sodium permeability Along this line of reasoning optimal conditions for cortical tissue in vitro would be such, in which a small volume of distribution of sodium is combined with low flux rate and low oxygen consumption That the maximal oxygen consumption may be misleading to the choice of medium electrolyte composition is indicated in the case of liver slices Attempts to optimize the medium with regard to oxygen consumption (Krebs 1950) has led to a saline medium which contains a far too high Na K ratio with special reference to glycogen synthesis (Hastings et al 1955) These latter investigators also showed a considerable loss of potassium when liver slices were incubated in a similar Ringers solution with high sodium concentration

It thus appears as if the problem of swelling of proximal tubular cells in vitro is closely linked to the problem of whether the process of reabsorption in the proximal tubules is confined to specialized, subcellular organells as suggested previously (Bojesen and Leyssac 1965) or whether the unidirectional net sodium transfer in vivo is caused by a sodium pump" localized at the basal membrane and passive transfer across a permeable luminal membrane as suggested by many authors The present technique in refined modification (cylinders of smaller diameters etc) might therefore contribute to the solution of this interesting problem

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Autoradiography of Injected Albumin-Bound 1-C^{14}-Palmitate in Pigeon Pectoralis Muscle

By

CLAES WIRSÉN

Received 20 January 1965

Abstract

Fat is considered to be the main fuel in sustained muscular work It is transported to the muscle in the form of free fatty acids (FFA) released into the blood from the adipose tissue and bound to plasma albumin The uptake of FFA in skeletal muscle as in other tissues is believed to be dependent mainly on concentration and on plasma flow, whereas nutritional factors seem to determine the extent to which the FFA taken up by the cells are oxidized or stored Available data indicate that, in the resting muscle, most of the FFA are incorporated into triglycerides or phospholipids and stored in the intracellular fat pool (Havel *et al* 1962, Göransson and Olivecrona 1964) Histochemical investigations suggest however, that the uptake and storage of fatty acids may not be equal in all muscle fibers Fat droplets are most numerous in fibers with a high content of oxidative enzymes (Nachmias and Padykula 1958, George and Naik 1958) The skeletal musculature of higher vertebrates is composed of three types of fiber, namely, *red*, *intermediate* and *white* fibers (cf Ogata and Mori 1964) which also seem to develop as three distinct populations in the fetus (Wirsén and Larsson 1964) During enhanced FFA mobilization as provoked by noradrenaline infusion, the three types become easily distinguishable owing to the different degrees of lipid deposition in the sarcoplasm (Carlson, Liljedahl and Wirsén 1965 Maunsbach and Wirsén 1965) This lipid deposition is generally correlated to myoglobin thus justifying the contention that most fat is deposited within red fibers (Carlson *et al* 1965)

However, the distribution of stainable lipid in a tissue section is no definite proof of a heterogeneous uptake of FFA The fatty acids may have been converted to compounds not visualized by the histochemical procedure Therefore, a method that makes it possible to follow the fatty acids themselves should be applied, and the histological picture compared with the results thus obtained It was decided to try the autoradiographic method developed by Ullberg (1954), including freeze-sectioning of unfixed tissue, thus preventing losses of fat-soluble labeled compounds

The pectoralis muscles of the pigeon were chosen as experimental material Their bio- and histochemistry have been rather extensively investigated by George and his group (see for instance George and Vallyathan 1964), and the fiber types are easily distinguished, *i e*, by their different diameters, the white fibers being very large and often situated at the border of the muscle bundles or in small groups among the very narrow red fibers As the pigeon is a small animal, moderate isotope dosages can be used to obtain a high activity in the tissue

Material and methods

Young healthy laboratory pigeons (*Columba livia*) were fasted overnight Water was given *ad libitum* 1 C[14] palmitic acid (Radiochemical Centre, Amersham, England), specific activity

3 min two after 6 and two after 15 min by a rapid injection of 2 ml of a 6 % solution of Mebumal® sodium The pectoral skin was rapidly removed and small pieces from the pectoralis major and minor (15-min animals) muscles were taken out and immediately frozen in liquid nitrogen They were then transferred to small aluminum boxes chilled with dry ice, and

nitrogen or fixed in formol sucrose solution and stained for succinic dehydrogenase, lipids, phosphorylase and glycogen as described elsewhere (Carlson *et al* 1965)

Results

white type In the 3-min experiments the distribution of isotope was somewhat diffuse,

although regions with white fibers showed a distinctly weaker activity The difference between red and white fibers became further accentuated in the 6-min and 15-min experiments, the white fibers showing only slightly more activity than the background still at 15 min after the injection of label The resolution of the autoradiograms was estimated to around 20 μ, and even single white fibers could be easily identified (Fig 1 A—D) In the pectoralis minor there were small areas of more intense blackening on a background of mixed moderate to weak activity (Fig 1 F) It turned out to be technically difficult to stain the pertaining tape sections so as to give a good picture of the distribution of fiber types However, a Sudan-stained section from an untreated animal shows that in the pectoralis minor the red fibers lie in small groups between intermediate and white fibers, much as is suggested by the autoradiogram (Fig 1 F) The histochemical stainings confirmed earlier observations of the high succinic dehydrogenase activity and fat content of red fibers and of the high phosphorylase activity and glycogen content of the white (George and Naik 1958, George and Scaria 1958, Dubowitz and Pearse 1960, Ogata and Mori 1964) These observations were also extended by the present finding that in the pectoralis minor medium sized fibers of the intermediate type occur besides the broad white and narrow red fibers

Discussion

The results of the present experiment clearly show that it is possible to study the distribution of injected fatty acid with a morphological method The contention that FFA are taken up by skeletal muscle in the resting state (Havel *et al* 1962, Goransson and Olivecrona 1964) is here confirmed and visualized Distinct differences are shown in the distribution of isotope between red and white and, possibly, also intermediate fibers of vertebrate skeletal musculature

However, to make justified an interpretation of this distribution pattern as due to a differential uptake of circulating FFA, certain requirements must be fulfilled First, the injected label should be incorporated in stable and poorly soluble compounds that remain in the cells for a considerably longer time than the biological half life of the label in plasma, so that the picture is not blurred by strong activity in the blood vessels Secondly, the elimination of label from the muscle fibers should be negligible compared to the influx of FFA from plasma, so that the distribution pattern is determined by the latter

That most of the FFA are incorporated in triglycerides and phospholipids also in the fasting animal has already been mentioned (Havel *et al* 1962), and at least in the rat the content of labeled palmitic acid is high in these compounds for the first 20 min (Goransson and Olivecrona 1964) The biological half life of plasma FFA in the pigeon is not known However, in the domestic fowl, it can be estimated to between a half and one minute (Carlson *et al* unpublished) The diffuseness of the autoradiograms 3 min after injection of label may in part be due to remaining plasma activity

Fig 1 A Autoradiogram from pectoralis major of pigeon 6 minutes after i v injection of 1 C^{14}-palmitate High activity in areas with red fibers Even single white fibers can be distinguished as white patches The broad interspace between black areas is due to white fibers lying in rows at the border of muscle bundles Exposure 6 weeks 15 ×
B The corresponding section The rows of white fibers at the border of muscle bundles have been slightly overstained Section thickness 20 μ Sudan Black B 15 ×



C Autoradiogram of longitudinally sectioned muscle fibers in the same preparation High activity in red fibers 40 ×
D The corresponding section. The red fibers are dark Sudan Black B 40 ×
E. Autoradiogram from pectoralis minor 15 minutes after injection of 1 C^{14} palmitate Small areas with high activity (black) on larger with moderate (grey) or weak (light grey) activity Exposure 15 weeks section thickness 20 μ 15 ×
F Pectoralis minor from untreated animal Three types of fiber small dark (red) medium sized with moderate staining (intermediate) and large with weak staining (white) Sudan Black B formol sucrose fixation 170

As regards elimination of fatty acid, the only way so far known for a muscle fiber would be oxidation However, in the pigeon, the content of succinic dehydrogenase an essential component in the fatty acid oxidation system, is several times higher in the red fibers, which display the highest radioactivity Thus the difference in amount of labeled fatty acid contained in red and white fibers respectively cannot be attributed to more rapid oxidation in the latter, but is most likely to depend on a different uptake

The relative amount of fat droplets characteristic of each fiber type in the pigeon muscle reflects, accordingly, the uptake of circulating FFA As the same types of fiber are found as well in other species from fishes to man (Ogata and Mori 1964), this *correlation may be valid for skeletal muscle of higher vertebrates in general As the* white fibers have a high glycogen content and seem to depend more on glycolysis than do the red fibers with their higher capacity for lipolysis and oxidation, the attractive hypothesis arises that each fiber receives—or extracts—the appropriate kind of fuel from the circulating blood The more scanty vascularization of white fibers is at least not likely to explain entirely the difference in label content, since the activity in them is far lower than would be expected should the blood flow alone account for the lower uptake Less sarcoplasm in white fibers than in red might be of importance in the pigeon, however, in the dog such a difference in the extension of sarcoplasm as could account for the variations in the number of fat droplets has not been found (cf Maunsbach and Wirsén 1965) Rather, the characteristic enzyme pattern of each fiber type suggests that the uptake of FFA from plasma proceeds at different rates in cells with different metabolic qualities

The present results thus support the concept of a selective uptake of plasma FFA if not possibly a selective extraction



autoradiographic technique

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The Distribution in the Mammal Body of F^{18} and P^{32} from Double-Labelled Na_2PO_3F

By

YNGVE ERICSSON and LARS HAMMARSTRÖM

Received 23 January 1965

Abstract

Ericsson Y and L Hammarstrom *The distribution in the mammal body of F^{18} and P^{32} from double labelled Na_2PO_3F* Acta physiol scand 1965 *65* 126—137 — Autoradiographic and quantitative investigations with F^{18}, P^{32} labelled Na_2PO_3F were performed on mice and rats in order to study the fate of the monofluorophosphate ion in the mammal body Comparisons were made with the distribution of F^{18} as fluoride ions and P^{32} as orthophosphate ions Intravenous injection of the double labelled PO_3F ion gave strikingly high concentrations of F^{18} in the intestinal lumen and of P^{32} in the intestinal wall A comparatively high concentration of F^{18} was found in the liver following the ingestion as the PO_3F ion Fluorine ingested as the monofluorophosphate ion did not pass the placenta to any greater extent than when given as the fluoride ion The indications of these data for further studies of the site and mechanism of splitting and toxicity of monofluorophosphate are discussed

Sodium monofluorophosphate Na_2PO_3F, is a fluorine compound which has attracted great interest in later years owing to its chemical and physiological properties It is easily soluble stable in water solution and not easily precipitated by calcium ions It is readily absorbed in the intestine but has a low acute toxicity in spite of the fact that its fluorine content is split off in the body to a great extent (Ericsson Santesson and Ullberg 1961 Sodium monofluorophosphate has a caries preventive action when applied to the tooth surfaces, evidently by a mechanism different from that of simple fluoride ions Ericsson 1963 and it is being used for different forms of local fluoride application to the teeth It has also been suggested as a suitable fluorine compound for the fluoridation of foods e g salt or flour and it might be used instead of sodium fluoride in the treatment of acute osteoporosis as in Paget s disease

The properties and uses of sodium monofluorophosphate seemed to warrant a closer investigation of the distribution of the PO_3F ion or its splitting products in the mammal body This was performed in the investigation to be reported by the use of P^{32}, and or F^{18} labelled Na_2PO_3F in autoradiographic experiments on mice and quantitative experiments on rats and mice

I. AUTORADIOGRAPHIC STUDIES

Methods

P^{32}, F^{18}-labelling of Na_2PO_3F

... P^{32} and F^{18} has been described earlier (Erics-

... orthophosphate ions Evaporation. Heating

... NH_4F Evaporation gives NaF labelled with

3 Fusion of $NaP^{32}O_3$ and NaF^{18} in stoichiometric proportion at 700—800° gives P^{32}, F^{18}-labelled Na_2PO_3F

Double labelling on a microscale with a quantity of Na_2PO_3F carrier small enough not to cause toxic symptoms on intravenous injection requires specially meticulous procedures, and the method used by us is therefore given in detail below

Solutions

A 138 mg $NaH_2PO_4 \cdot H_2O$ dissolved in 1 25 ml distilled water
B P^{32} solution containing a microcurie quantity of P^{32} in about 0 025 ml solution
C 42 mg NaF dissolved in 1 25 ml distilled water

P^{32}-labelling of $NaPO_3$

residue is heated to 530° for 5 hrs

F^{18} production

$LiNO_3$ enriched to about 96 % with Li^6, is neutron irradiated in the uranium reactor which gives H^3 and F^{18} as radioactive products (Ericsson 1961) The irradiated salt is dissolved in distilled water and calcium phosphate is precipitated in the solution F^{18} is taken up practically quantitatively in the precipitate The precipitate is washed twice with distilled water to remove H^3 and radioactive contamination mainly Na^{24}, and is then quantitatively transferred to a micro-distillation apparatus and distilled with about 40 % $HClO_4$ using a slow air stream About 5 ml distillate is taken up in an empty receptacle The distillate is evaporated in a platinum crucible to about 0 025—0 040 ml.

F^{18}-labelling

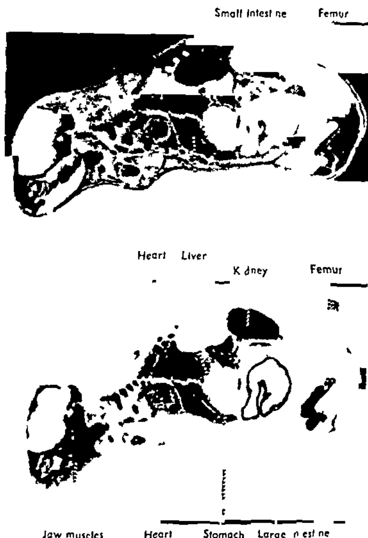
In the quartz tube containing P^{32} labelled $NaPO_3$, 0 025 ml solution C is pipetted from a microburette through a capillary Careful evaporation The distillate which has been concentrated according to the description above is transferred to the quartz tube and evaporated to complete

Chemical identity and purity tests

indicated a monofluorophosphate purity above 95 per cent

Autoradiographic technique

The double-isotope autoradiographic technique followed the same principles as have been described earlier by Appelgren *et al* (1961) Albino mice weighing about 30 g and one mouse



F g 1 Distr bution of F^{18} (above) and P^{32} (below) 5 m nutes af er v nject on of double-labelled Na_2PO_3F

in advanced pregnancy we gh ng 45 g were injected in a ta l vein the dose g en was 2 μc P^{32} and about 0 75 mc F^{18} n 0 5 ml 30-mM solut on of Na_2PO_3F This dose of Na_2PO_3F had been found to produce no tox c symptoms in m ce

... mouse and 5 ... in a ... sed ... were ... ere ... od ... ar

chemical fogg ng dur g t e ... xposu e ... a exp ...

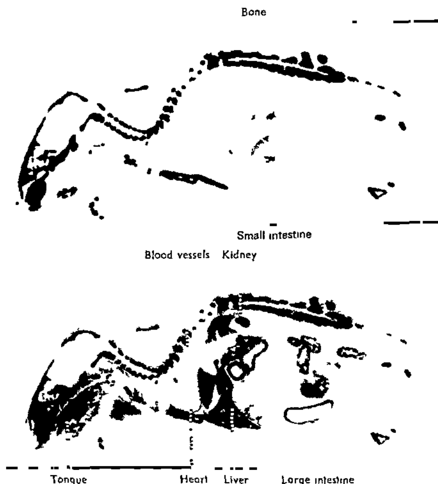


Fig 2 Distribution of F^{18} (above) and P^{32} (below) 20 minutes after i v injection of double labelled Na_2PO_3F

ried out in the cold (— 10°) The sections were then dried in the cold (— 10°) and after a delay of 2 days during which time the F^{18} content desintegrated a new exposure was made which gave the P^{32} autoradiograms after 5—6 weeks

A 5-hour exposure performed after the decay of F^{18} gave no detectable autoradiographic blackening which proved that the P^{32} did not interfere noticeably during the registration of F^{18}

For comparison with the autoradiographic Na_2PO_3F experiments P^{32} was also injected i v as low-carrier orthophosphate in two mice survival time 5 and 60 min respectively

Results

Autoradiography of F^{18} + P^{32} as monofluorophosphate

Fig 1—3 demonstrate the distribution of F^{18} and P^{32} from the double labelled PO_3F ion after 5 20 and 60 min survival time respectively



Fig 3 Distribution of F^{18} (above) and P^{32} (below) 60 minutes after i v injection of double labelled Na_2PO_3F

Already 5 min after the i v injection of $Na_2P^{32}O_3F^{18}$ differences were clearly observable in the distribution patterns of the two isotopes these differences appeared to be more accentuated with time The main tendency was that F^{18} was more rapidly and selectively taken up by the *skeleton* than P^{32}

The concentrations in the *blood* of both F^{18} and P^{32} rapidly decreased and were very low 20 min after injection



Fig 4 Distribution in the kidney of F^{18} (left) and P^{32} (right) 5 min after i v injection of double-labelled Na_2PO_3F

Bone Liver Placentae

Heart Intestine Foetal skeletons

Bone Liver Spleen Placentae

Heart Intestine Foetal skeletons

Fig 5 Distribution of F^{18} (above) and P^{32} (below) in a pregnant mouse 30 min after i v injection of double labelled Na_2PO_3F

The F^{18} concentration in the *liver* was variable but appeared to follow the concentration in the blood The P^{32} concentration of the liver also equalled that of the blood after 5 min but then considerably increased The same conditions were observed in the *spleen* where the P^{32} was mainly localized in the area of the marginal sinuses.

In the *intestine* the distribution of the two isotopes greatly differed already 5 min after injection F^{18} was mainly found in the lumen of the small intestine and in some



Fig 6 Distribution of P^{32} 60 minutes after i v injection of single labelled Na_2PO_3F

crypts of the large intestine while the concentration was low in the intestinal wall. In contrast to this P^{32} was strongly concentrated in the wall of the large intestine and to a lesser degree in the wall of the small intestine

Some accumulation of P^{32} was seen in the *myocardium* and *skeletal muscles* while F^{18} was never seen in appreciable concentration in these tissues

In the *kidney* F^{18} was mainly concentrated in the hilus while P^{32} was also accumulated in the cortical area (Fig 4)

In the *skin* a distinct double contour of both isotopes is observed especially at the 5 min interval following the injection probably reflecting the localization of blood vessels

Fig 5 demonstrates the F^{18} and P^{32} distribution in a *pregnant mouse* 30 min after the injection of double-labelled Na_2PO_3F. The mineralized parts of the foetal skeletons and some small spots in the placentae show a concentration of both isotopes The maternal liver and intestines show the same distribution picture as described above

Autoradiography of P^{32} as orthophosphate

The distribution of i v injected orthophosphate P^{32} was found to be similar in some respects to that of P^{32} after injection as the PO_3F ion For example Fig 6 demonstrates a high concentration in the liver and fairly high concentrations in the spleen myocardium and skeletal muscles after one hour

II QUANTITATIVE STUDIES

The notable concentrations of P^{32} and in some cases also F^{18} in the liver of the mouse that were observed autoradiographically following an i v injection of double labelled Na_2PO_3F warranted a quantitative investigation of the uptake in the liver of these isotopes in comparison with blood kidney and bone The placental transfer of the double label was also thought to deserve a quantitative study Finally an effort was made to ascertain as far as possible that the F^{18} and P^{32} concentrations appearing autoradiographically in the intestinal lumen and the intestinal wall respectively were in agreement with the data of a quantitative experiment

Methods

Liver uptake, compared to concentrations in blood, kidney and bone

phosphate

Double-labelled Na_2PO_3F, prepared as described earlier (Ericsson 1961) was dissolved in milk to give a 2 5-mM concentration of monofluorophosphate 2 5-mM NaF in milk was prepared in an analogous way after F^{18} labelling of NaF P^{32} labelling was done by simply adding a P^{32} solution, as low-carrier orthophosphate, to milk since ordinary cow's milk contains over 20-mM P per litre

chlorethylene and opened and blood was drawn from the heart with a heparinized syringe, the animal was then immediately sacrificed by stretching the spine, one kidney and part of the liver were taken out and one femur dissected free from soft tissue

$F^{18} + P^{32}$, in the case of Na_2PO_3F, and for F^{18} in the case of NaF In the former case a second radiometric analysis was performed after the decay of F^{18}, the P^{32} activity was thus obtained and F^{18} could be calculated as the difference between the first count and the P^{32} count. Every analysis involving F^{18} naturally had to be timed on the minute and recalculated to a standard time

Placental transfer

orthophosphate buffer to the same number of mice

Four mice in each experiment were sacrificed after 1 hr survival time, the other four after 4 hrs. Blood and liver samples were taken as in the rat experiment described above, and three foetuses and three placentae from each mouse were taken for analysis which was performed as with the rat samples

Distribution of double label of Na_2PO_3F in intestinal wall and contents

was then overstretched and the stomach small intestine, large intestine spleen and part of the liver removed The contents of the three parts of the gastro-intestinal tract were squeezed out as completely as possible Each specimen was placed in a plastic tube and counted as described above

The calculations of the quantitative double-tracer experiments were carried out in an IBM 1401 electronic computing machine

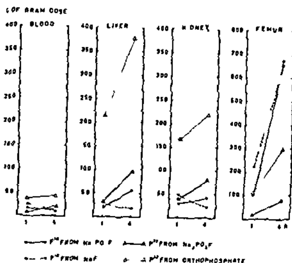


Fig 7 F^{18} and P^{32} in blood liver kidney and femur of rats 1 and 4 hours after ingestion of labelled compounds with milk

The ordinates give the contents per gram blood or tissue as percentages of the dose given per gram animal For details see text

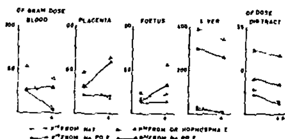


Fig 8 F^{18} and P^{32} in blood placenta foetus liver and digestive tract of mice 1 and 4 hours after ingestion of labelled compounds in water solution

For the digestive tract the ordinate gives the percentage of the total dose remaining in the total tract For the other specimens the denotions are the same as in Fig 7 Further details in text

Results

The average results of the *rat experiments* appear from the diagram Fig 7 Calculations on the individual data showed that F^{18} and P^{32} given as Na_2PO_3F were both significantly more concentrated in the liver than in the blood after 4 hrs and even more than in the kidney after 4 hrs This was in marked contrast to F^{18} derived from NaF which was about the same in the liver as in the blood and significantly lower in the liver than in the kidney at both time intervals

The liver concentration of P^{32} from orthophosphate was still much higher even higher than that of the femur

The results of the *mouse experiments on placental transfer* are condensed in Fig 8

In these experiments where the fluoride phosphate compounds were given in water solution the absorption was notably complete already after 1 hr especially from Na_2PO_3F This should be the explanation of the generally decreasing values in blood and liver from 1 to 4 hrs in contrast to the corresponding values in the rat experiments

The placental concentrations of F^{18} and P^{32} from Na_2PO_3F were significantly lower than from NaF + orthophosphate at 1 hr differences that had levelled out after 4 hrs just as in the blood

TABLE I Distribution of F^{18} and P^{32} from Na_2PO_3F after i v injection on mice

Per cent of gram-dose/gram organ ± standard error

	F^{18}		P^{32}	
	5 min	20 min	5 min	20 min
Stomach	50.9±2.4	25.4± 3.2	43.8± 3.3	48.0±2.7
content	10.2±0.6	5.1± 0.5	17.6± 0.7	13.8±2.2
Small intestine	65.4±2.2	33.5± 1.7	61.8± 5.8	70.0±6.5
content	63.6±7.8	79.4±10.2	22.4± 0.6	28.9±7.6
Large intestine	61.0±2.6	33.5± 0.7	102.7±13.4	110.0±6.3
content	17.2±1.1	30.2± 2.3	31.1± 4.4	66.0±1.5
Blood	92.5±9.2	26.4± 6.9	65.1± 6.4	26.9±1.8
Liver	65.4±3.7	31.5± 1.0	64.5± 4.4	79.7±2.8
Spleen	94.7±3.4	40.6± 1.3	124.1± 4.9	157.8±6.6

The foetal concentrations of F^{18} and P^{32} were throughout lower than those of the placenta after ingestion as Na_2PO_3F, while this difference was found only at the 1 hr interval following ingestion as NaF + orthophosphate

F^{18} from Na_2PO_3F had about the same concentration in the liver as in the blood after 1 hr but was much higher in the liver after 4 hrs F^{18} from NaF showed a significantly lower concentration in the liver than in the blood after 1 hr, while the difference was small after 4 hrs

P^{32} from both Na_2PO_3F and orthophosphate was many times higher in the liver at both time intervals

The results of the *mouse experiments on the distribution of the double label in the intestinal walls and contents* appear from Table I and Fig 9 Table I also includes the figures simultaneously obtained for blood liver and spleen concentrations

The diagram demonstrates particularly two features which confirm the autoradiographic pictures of the distribution of F^{18} and P^{32} from i v injected Na_2PO_3F the high F^{18} concentration in the contents of the small intestine and the high P^{32} concentration

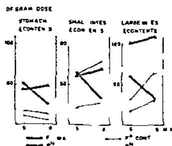


Fig 9 F^{18} and P^{32} in walls and contents of digestive tract segments 5 and 20 min after i v injection as double-labelled Na_2PO_3F in mice

The ordinates graduated as in Fig 7 further details in text

in the wall of the large intestine Owing to the impossibility of separating quantitatively the walls and contents of the intestines the true differences between the isotope concentrations of these parts must be assumed to be greater still

Table I further demonstrates a high concentration of P^{32} in the liver and particularly in the spleen, both increasing from 5 to 20 min after the injection F^{18} shows some concentration in the spleen after 20 min

Discussion

The results as regards the distribution in blood and femur of F^{18} and P^{32} from Na_2PO_3F agree on the whole well with previous data (Ericsson *et al* 1961)

The widely different uptake of P^{32} and F^{18} in the femur during 4 hours following the ingestion of Na_2PO_3F indicates that the PO_3F ion is generally not taken up as an entity by the bone mineral in vivo, which has been found to be the probable mechanism for the reaction in vitro of PO_3F with hydroxy apatite and dental enamel (Ericsson 1963)

The earlier unknown concentration of both isotopes in the liver after administration as PO_3F ion was established by the quantitative experiments on rats and at the 4 hr interval in the mice The mechanism of the storage in the liver and the fate there of the PO_3F ion is, however, still unknown The simultaneous accumulation in the spleen makes it an obvious question whether the reticulo-endothelial system is involved in this storage

The total fluorine concentration in the liver will, for a certain length of time following ingestion of the PO_3F ion, be considerably higher than following the administration of F ions Our present lack of knowledge of the mechanism and site of monofluorophosphate splitting in the body makes it difficult to interpret this finding Individual variations in the splitting rate of the monofluorophosphate ion are possible, and, since the micro-labelling technique did not permit tests on the chemical purity of every batch, variations in this respect are obviously not excluded However, our purity tests and the chemical behaviour of monofluorophosphate produced by the same method for a number of other investigations (Ericsson 1961, and previously quoted papers) indicate that the variation in purity should be rather limited

Owing to the temporary concentration of fluorine from Na_2PO_3F in the liver it is *conceivable that the chronic toxicity of this compound might* be as great as or greater than that of the same fluorine quantity as fluoride ions This possibility has been the object of a special study in rats, which has demonstrated a similar, although apparently slightly lower, chronic toxicity of the fluorine content of Na_2PO_3F compared to that of NaF (Poulsen and Ericsson, to be published)

The concentration of P^{32} from the PO_3F ion in the intestinal wall and of F^{18} in the [illegible] fact that [illegible]

[illegible]

[illegible]

found F^{18} fluoride in the stomach only in one case where it could be suspected that [illegible] animal had been licking its urine during the survival time

Our theory is that this appearance of P^{32} and F^{18} in the intestinal wall and *lumen*, respectively, is related in some way to the metabolic mechanism of the PO_3F ion An enzymatic splitting of this ion may for example, be concentrated in the intestinal wall

The authors acknowledge the valuable advice and criticism of Assoc Professor Sven Ullberg and able assistance of several members of the staffs of their institutions in particular that of Mr Olof Ekberg Chem Engineer

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Recurrent and Reflex Discharges in Plantar Muscles of the Cat

By

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Received 27 January 1965

Abstract

Gassel M M and M Wiesendanger *Recurrent and reflex discharges in plantar muscles of the cat* Acta physiol scand 1965 *65* 138—142 — The response subsequent to the maximal direct response in plantar muscles of the cat elicited by electrical stimulation of the tibial nerve at the ankle had an early, triphasic, and a late, polyphasic, component Deafferentation of the hind leg showed that the early component was an antidromically induced recurrent discharge and the late component a polysynaptic reflex. A monosynaptic reflex could however add to or even replace the recurrent discharge at the same latency as the recurrent discharge when the strength of stimulation was reduced or following repetitive stimulation As to the secondary response in the intrinsic hand and foot muscles in man (F-wave) it is unsettled whether the first deflection originates from a recurrent discharge or is a combination of a recurrent discharge and a monosynaptic reflex This makes the F-wave unsuitable for the measurement of sensory conduction velocity

In man Hoffmann 1918) described a secondary response in muscle to electrical stimulation of the mixed nerve with a longer latency than the direct response The secondary response was assumed to be a monosynaptic reflex equivalent to the tendon reflex In the calf muscles the secondary response (H-wave), elicited by stimulation of the popliteal nerve, appears at a lower threshold than the direct muscle response, the amplitude is decreased when a significant fraction of motoneurones is invaded antidromically The secondary response recorded in the anterior tibial muscle and in the intrinsic muscles of the foot and hand have different properties (F-wave The secondary response has a higher threshold than the direct muscle response with greatest amplitude at maximum stimulation for the direct response Magladery and co-workers (1950 1951 interpreted this secondary response F-wave as being a reflex transmitted by sensory fibres with a

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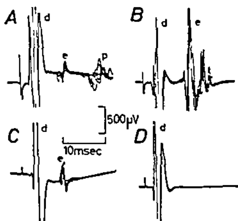


Fig 1 Secondary response in plantar muscles of cat to stimulation of the tibial nerve

ondary discharge
B Stimuli submaximal for the direct response (d) Note increase of the early component (e) of the secondary response
C Secondary response to supramaximal stimuli for the direct response after deafferentation consisting only of the early component (e)
D Section of the nerve proximal to the site of stimulation, the secondary response has disappeared

higher threshold and a lower conduction velocity than the motor fibres Renshaw (1941) however demonstrated by leading off from muscle nerves after section of the dorsal roots that an antidromic volley elicited a centrifugal recurrent discharge in 2—3 % of the motor fibres with a central delay comparable to a monosynaptic reflex Dawson and Merton (1956) referring to Renshaw's paper came to the conclusion that the F wave is a recurrent discharge and has nothing to do with a proprioceptive reflex This assumption was supported by the finding of the same conduction velocity for the F-wave and the direct motor response

To investigate whether the secondary response is a recurrent discharge or is composed of a recurrent discharge and a reflex we have compared the potential shape, latency and amplitude of the secondary response in intact and deafferented hind legs of cats

Material and methods

anesthesia and to simulate the usual conditions of testing in humans Four cats were examined during the deafferentation under Nembutal® anesthesia In 2 the response from both hind legs was recorded before and after the extradural section of each dorsal root, and 2 were tested *before and after intradural section of all dorsal rootlets*

The recording device consisted of concentric needle electrodes 0 7 mm in diameter, differential amplifiers with a frequency response of 3 db down at 10 000 and 30 cps and a dual beam oscilloscope from which single sweeps and superimposed sweeps were photographed Rectangular pulses of 0 1 msec duration were delivered from a stimulator with a double screened output transformer through 2 steel needles inserted percutaneously along the nerve

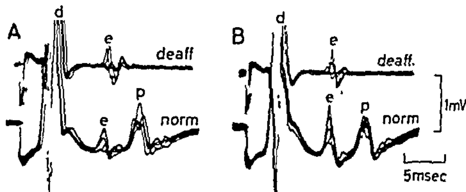


Fig 2 Secondary response in the plantar muscles of cat to stimulation of the tibial nerve at the ankle, 5 superimposed sweeps Upper trace deafferented side (deaff), lower traces non-deafferented side (norm)
A before, B 5 sec after repetitive stimulation (200 p s) Note increase of early component (e) on the non-deafferented side (norm) in B

Results

The effect of chronical extradural and intradural deafferentation has been described (Wiesendanger 1964) Deafferentation caused hyperactivity of extensor muscles, most pronounced after intradural deafferentation, indicating an increase in excitability of motoneurones

The secondary response of normal hind legs

Supramaximal stimuli for the direct response to the tibial nerve at the ankle elicited a secondary response with two components in the plantar muscles (Fig 1 A) The first component was triphasic with a latency of 8 4 ± 1 1 msec (n = 16) In the individual animal the latency did not vary more than 1 msec The amplitude ranged from 0 2 to 1 mV *The late component had a latency of 12 1 ± 1 5 msec (n 16)*, this potential was polyphasic and varied in shape and amplitude from stimulus to stimulus even when the rate of stimulation was reduced from one per second to one every five seconds, the amplitude (0 1 to 3 2 mV) and the shape depended on the position of the recording electrodes In five of seven cats supramaximal stimulation of the nerve with trains of five seconds at a frequency of 200—250/sec was followed by a potentiation of the early component of the secondary response, the response to five stimuli at a rate of one per second being two to five times greater after than before tetanization (Fig 2)

When the strength of stimulation was reduced to submaximal values for the direct response, the amplitude of the secondary response could increase (Fig 1 B) or decrease and the shape varied such that it was no longer possible to distinguish an early and a late component

The secondary response of chronically deafferented hind legs

In chronically deafferented muscles the secondary response appeared only when the stimulus was near maximum for the direct response Its shape, latency 8 1 ± 1 2 msec) (n = 16) and amplitude (0 1—0 5 mV were about the same as those of the early com-

ponent in normal hind legs (Fig 1 C) There was no second component in the secondary response after deafferentation (See Fig 1 and 2) The first component was present immediately after deafferentation except in one animal in which the operation was traumatic with severe hemorrhage In this animal the secondary discharge was normal on the day after operation

The central origin of the secondary discharge was indicated (1) by a shorter interval between the direct and the secondary response when the popliteal nerve (interval = 4 4 msec) was stimulated instead of the tibial nerve at the ankle (interval = 6 2 msec), and (2) by the absence of the secondary response after the nerve was cut proximally to the site of stimulation (Fig 1 D) The following observations point to backfiring within the motoneurones as cause of the secondary response rather than to a synaptic pathway tetanization caused no potentiation, in 8 cats the amplitudes of the secondary response before tetanization ranged between 0 1 and 0 5 mV and after tetanization between 0 1 and 0 4 mV (Fig 2) Similarly the amplitude remained unchanged when the frequency of stimulation was increased from 0 3 to 30 per second (tested in 3 cats) This is unlike a reflex response elicited by an electrical stimulus to the mixed nerve which antidromically (Renshaw 1941) and orthodromically (Lloyd 1943) affects the motoneurone discharge of subsequent reflex volleys

The roots which contribute to the secondary response in the plantar muscles

When the ganglia were prepared extradurally or the dorsal rootlets exposed intradurally the secondary response in Nembutal® anesthesia had an early component with the same range of latencies (8 7 ± 0 2 msec) as in normal hind legs without anesthesia the amplitude ranged between 0 5 and 1 7 mV The early component was followed by a discharge of complex shape The secondary response remained unchanged when the dorsal roots L4 L5 L6 and L7 were cut extradurally (two cats) section of the root S1 abolished the late component The secondary response on the non-operated side was unchanged

Discussion

The secondary response of plantar muscles to supramaximal stimulation of the mixed nerve had two components The latency of the first potential was compatible both with a monosynaptic and a recurrent discharge (Renshaw 1941 Lloyd 1943) the latency of the late component indicated a polysynaptic pathway Deafferentation abolished the late component but not the early component indicating that the remaining early discharge in deafferented muscles is a recurrent discharge That the change in the secondary response after deafferentation is hardly due to a change in central excitability is evidenced by the finding of the same range of amplitude and latency of the early component on the normal and the deafferented side Neither can the disappearance of the late component be explained by a depression of excitability due to deafferentation since this procedure was associated with hyperactivity in extensors considered to indicate a state of central hyperexcitability Thus makes it possible that the early component of the secondary response evoked by supramaximal stimuli in normal cat plantar muscles is an antidromically induced recurrent discharge However depending on the strength and rate of stimulation there are conditions in which a monosynaptic reflex is superimposed on a recurrent discharge or even may replace it This is evidenced by an increase in the early component when the stimulus is sub-

maximal for the direct response or when the nerve was stimulated repetitively before the test responses In man the type of response similar to that studied in cats is the F wave, the threshold of which is higher than that of the direct response As in cats the secondary response elicited with supramaximal stimuli has the same range of amplitudes and is variable from stimulus to stimulus, so much that not every stimulus evokes a secondary discharge The latency is compatible with both a monosynaptic reflex and a recurrent discharge Tetanization causes a potentiation of the amplitude (Hagbarth 1962) Unlike the results in muscles of the cat, a later polyphasic component of the secondary response was not found in human muscles by Magladery and McDougal (1950) If it is a recurrent discharge, it is obviously unsuitable for the measurement of sensory conduction velocity as proposed by Magladery and McDougal (1950) and Liberson (1962)

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Steady State Diffusing Capacity during Prolonged, Non-Steady State Sitting Exercise in Ordinarily Trained Young Men

By

LARS GORAN EKELUND and ALF HOLMGREN

Received 26 January 1965

Abstract

[illegible]

creased only slightly The resulting increase in the dead space [illegible]

Earlier studies of steady state diffusing capacity ($D_{L_{CO}}$), have demonstrated that during exercise $D_{L_{CO}}$ varies approximately hyperbolically with oxygen uptake ($\dot{V}_{O_2}$) (Freyschuss and Holmgren 1965, Holmgren 1965c) The changes above a $\dot{V}_{O_2}$ of 40 per cent of the maximum oxygen uptake were only slight, especially in untrained subjects (Holmgren 1965c) For this reason $D_{L_{CO}}$ during exercise at a load increasing $\dot{V}_{O_2}$ to more than 40 per cent of $\dot{V}_{O_2\ max}$ or increasing heart rate to or above 120 beats per minute was regarded as an estimate of the maximum diffusion capacity of the individual During prolonged non steady-state exercise Ekelund and Holmgren (1964) found that when a subject worked at a constant load, the cardiac output remained constant, the heart rate increased and the stroke volume decreased Simultaneous measurements of

the physiological dead space of the lungs (V_D) showed that this increased, which was interpreted as a result of altered ventilation-perfusion relationships (Severinghaus and Stupfel 1957) The authors suggested that the falling stroke volume and underperfusion of part of the lung could be explained *by orthostatic blood shifts within the capacitance vessels* It seemed probable that the upper part of the lung was underperfused (cf West 1963)

The present investigation was undertaken to try to establish whether such non steady-state exercise would influence the D_{LCO} as measured by the modified steady state technique (Filley, McInthosh and Wright 1954, Linderholm 1957) and to monitor significant variables such as the blood gas tensions, pH and the standard bicarbonate in arterial blood

Material and methods

The study was performed on 6 healthy young men without history, symptoms or signs of pulmonary disease The chest X-ray was normal in all subjects

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Calculations The D_{LCO} was calculated as the quotient between the carbon monoxide uptake ($\dot{V}_{CO}$) and the driving pressure, alveolar CO pressure minus mean capillary CO pressure ($P_{A_{CO}} - P\bar{c}_{CO}$) The $\dot{V}_{CO}$ was calculated with the aid of the equation published by Filley et al (1954) The $P_{A_{CO_2}}$ was taken to equal $P_{a_{CO_2}}$ The $P_{A_{CO}}$ was calculated by means of the Bohr equation, assuming that the dead space for CO was the same as that for CO_2 The $P\bar{c}_{CO}$ was calculated according to Linderholm (1957) For all other derived data (Table II) conventional equations were used All calculations were programmed for an IBM computer 1401

The *electrocardiogram was recorded at rest and during work*

tion according to the method described

concentration Hb g 100 ml was determined spectrophotometrically as ... static *spirometry* with determination of vital capacity and functional residual volume was performed by helium dilution in a closed system

Procedure

The experiments were planned as follows Exercise should be performed in sitting position at a load that increased the heart rate above 120 beats per minute after 6 min work and increased the heart rate slowly during one hour's work to or above 170 beats per minute The D_{LCO}, expired gas volumes and arterial gas tensions were measured after 6 10 30 and 60 min exercise

To attain this each volunteer was subjected to a pilot test including work at successively increasing loads up to a heart rate of 140 150 beats per minute At the load at which this heart rate was reached they were requested to work for one hour With the aid of this pilot test the load for the actual experiments was chosen

On the day of the experiment, including determination of alveolar gas exchange the subjects arrived at the laboratory early in the morning All had had a light meal before the experi-

TABLE I Anthropometric data in six healthy young men THb = total hemoglobin g W_{170} = rate of work that can be performed at a heart rate of 170 beats per min kpm/min, RV = *residual volume FRC = functional residual capacity, VC = vital* capacity and TLC = total lung capacity Respiratory volumes in l BTPS

Case	Age years	Height cm	Weight kg	Heart vol ml	THb g	Hb conc g/100 ml	Blood vol l	Sitting W_{170} kpm/min	Static spirometry: RV l, BTPS	FRC l, BTPS	VC l, BTPS	TLC l, BTPS
1	25	185	70	780	690	13 3	5 2	950	1 88	4 80	5 90	7 78
2	26	173	63	650	655	13 4	4 9	1 000	1 46	3 69	4 97	6 43
3	24	183	71	720	800	14 1	5 7	1 000	1 79	3 44	4 49	6 28
4	23	187	71	660	765	13 0	5 9	950	2 11	4 09	5 33	7 44
5	27	191	79	965	800	13 4	6 0	950	—	—	7 24	—
6	24	174	68	650	560	12 3	4 6	1 000	1 98	4 00	5 47	7 45

continuously with a thermocouple and the temperature read at the time of withdrawal was used to correct for differences between rectal temperature and the temperature of the cuvettes used for measuring P_{O_2} and P_{CO_2}

Results

The *anthropometric* data are presented in Table I The *age of the subjects varied between* 23 and 27 years The mean *height* was 182 cm, i e above the average and the mean weight 70 3 kg (SD ± 6 3) The *heart volume* was on an average 738 ml (SD ± 124 which lies on the regression line for heart volume and THb and heart volume and W_{170} reported with an identical technique by Freyschuss and Holmgren (1965) The *total amount of hemoglobin* averaged 712 g (SD ± 95) which is slightly higher in relation to weight than observed in a similar material of young men (Freyschuss and Holmgren 1965) but lower than in the normal material presented by Holmgren *et al* 1957) The *blood volume* averaged 5 4 l (SD ± 0 6) or 76 5 ml per kg body weight. The *Working capacity* expressed as W_{170} kpm min averaged 975 kpm/min (SD ± 20) in

TABLE II Respiratory data at rest supine and during long term exercise in sitting posit on in heimer (1950)

Case and time min	*Work load kpm/min*	*Pulse rate beats/min*	*Resp rate breaths/ min*	$\dot{V}_{O_2}$ *ml STPD/ min*	*RQ*	$\dot{V}_E$ *l BTPS/ min*
1	rest	63	10	324	0 73	7 7
10	750	131	14	1,464	0 90	32 5
30	750	160	20	1 759	0 90	39 1
53	750	173	22	1 816	0 86	40 0
2	rest	61	14	256	0 74	6 9
7	750	131	19	1 590	0 88	34 2
30	750	151	22	1 783	0 86	40 4
53	750	169	33	1 752	0 87	44 0
3	rest	77	16	335	0 81	8 9
6	800	135	27	1 844	0 99	45 3
30	800	156	30	1 959	0 95	51 0
60	800	159	33	2 019	0 95	52.3
4	rest	79	12	302	0 87	9 7
6	800	140	18	1 858	0 87	38 5
30	800	154	18	1 873	0 89	40 9
60	800	165	22	1 984	0 84	42 7
5	rest	65	16	318	0 77	8 0
7	800	146	22	1 899	0 88	41 6
31	800	165	24	1 973	0 91	47 2
60	800	173	25	2 181	0 88	52 6
6	rest	68	18	252	0 80	8 2
6–7	650	134	23	1 562	0 93	38 8
30	650	151	26	1 587	0 92	41 1
60	650	163	31	1 639	0 89	45 5

sitting position without any significant difference in lying position These values lie close to the regression line for W_{170} on heart volume and W_{170} on THb reported by Holmgren *et al* (1958 and Freyschuss and Holmgren (1965)

Static spirometry The values for residual volume functional residual capacity vital capacity and total lung capacity are presented in Table I All values fall within the normal range of variation reported with the same technique by Grimby *et al* (1963

Comment on the material The anthropometric analysis of the subjects used in the present study shows that they can be classified as fairly tall young ordinarily trained men, with an ordinary tendency to orthostatic blood shifts as judged by the increase in heart rate after 8 minutes standing and a comparison of the heart rate response during exercise in the sitting and supine positions.

six healthy ordinarily trained young men The symbols used are those suggested by Pappen

$\dot{V}_D$ ml BTPS	CO Hb %	$D_{L_{CO}}$ ml STPD/min/mmHg	$P_{a_{CO_2}}$ mmHg	$P_{a_{O_2}}$ mmHg	pH units	Stand bicarb mE/l
190	–	–	38 3	93	7 45	27 1
346	1 81	39 8	47 6	86	7 40	27 1
378	6 45	49 2	45 3	87	7 42	25 8
376	8 22	43 4	44 3	86	7 44	25 4
164	–	–	40 0	90	7 45	27 6
256	4 81	29 2	42 9	85	7 43	26 9
373	6 52	33 8	43 2	86	7 42	28 4
257	8 90	32 6	39 2	93	7 46	22 4
156		–	40 9	90	7 45	26 1
319	5 01	39 7	44 6	94	7 41	24 1
351	6 68	44 4	41 2	94	7 46	26 7
372	7 99	40 6	43 1	95	7 46	26 3
212	–		34 4	105	7 55	29 4
218	5 15	38 1	42 8	85	7 45	26 9
339	7 31	39 9	43 2	84	7 46	26 9
293	10 23	37 1	41 7	92	7 48	26 9
149	–	–	42 4	85	7 45	27 8
300	5 66	35 8	42 8	88	7 40	25 6
371	7 70	40 5	42 2	96	7 43	25 2
378	9 44	39 2	40 1	98	7 46	27 1
169	–	–	39 8	89	7 44	23 5
243	5 03	27 1	39 5	91	7 46	24 5
257	7 97	27 3	38.3	92	7 50	26 2
288	9 39	34 4	38 7	91	7 50	27 0

Observations during long term non steady-state exercise

Measured and derived data are presented in Table II The *work load* during the study varied between 650 and 800 kpm/min corresponding to an on average of 77 9 % of their W_{170} The *heart rate* (HR) increased after 7 min work to on an average of 136 beats per minute At 30 min the heart rate had risen further to 156 beats per minute and after 60 min to 167 beats per minute (SD ± 3 9) The *oxygen uptake* ($\dot{V}_{O_2}$) averaged 1703 ml STPD/min after 7 min 1 899 ml/min after 60 min i e there was a slow continuous increase (see Fig 1 2 and 3) of 11 5 per cent over the 7 min value The *ventilation* ($\dot{V}_E$) increased to 38 5 l BTPS/min after 7 min work Continued work caused $\dot{V}_E$ to increase further to 43 3 l/min after 30 min and 45 9 after 60 min work. The increase during the prolonged exercise averaged 19 2 per cent of the 7 min value This increase

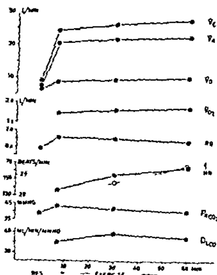


Fig 1

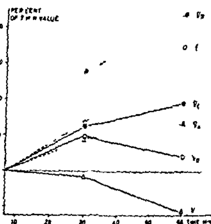


Fig 2

Fig 1 Respiratory data at rest and during exercise in six young men $\dot{V}_E$ = total ventilation l BTPS min^{-1}, $\dot{V}_A$ = alveolar ventilation, l BTPS min^{-1} $\dot{V}_D$ = dead space ventilation l BTPS min^{-1} $\dot{V}_{O_2}$ = oxygen uptake ml STPD min RQ = respiratory quotient HR = heart rate beats min f = respiratory rate breaths/min P_{aCO_2} = arterial carbon dioxide tension mm Hg and $D_{L_{CO}}$ = pulmonary diffusing capacity for carbon monoxide ml STPD min^{-1} mm Hg

Fig 2 Ventilatory responses during long term exercise Data are presented as a percentage of the values after 7 min work V_D = physiological dead space ml BTPS V_T = tidal volume ml BTPS Other symbols as in Fig 1

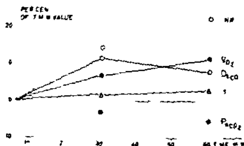


Fig 3 Respiratory data and heart rate during long term exercise Data given as a percentage of 7 min value T = rectal temperature °C Other symbols as in Fig 1

during exercise was effectuated by an increase in the respiratory rate f from 20 5 after 7 min to 27 7 breaths per minute after 60 min work in spite of a decrease in the tidal volume V_T from an average of 1720 ml BTPS after 7 min to 1648 ml BTPS after 60 min work The alveolar ventilation $\dot{V}_A$ l BTPS min calculated on the assumption of $P_{A_{CO_2}} \approx P_{a_{CO_2}}$ averaged 31 3 l BTPS min after 7 min corresponding to a $\dot{V}_A/\dot{V}_{O_2}$ of 18 4 During continued exercise $\dot{V}_A$ rose moderately to 34 1 l min after 30 min and 35 5 l BTPS min after 60 min This increase however essentially matched the increase in $\dot{V}_{O_2}$ at the same time which is expressed by the values for $\dot{V}_A/\dot{V}_{O_2}$ after 30 min

TABLE III Mean values for total ventilation, $\dot{V}_E$, l BTPS/min, alveolar ventilation $\dot{V}_A$, l BTPS/min, dead space ventilation, $\dot{V}_D$, l BTPS/min, physiological dead space, V_D, ml BTPS, tidal volume, V_T, ml BTPS and respiratory rate, f, breaths per min during prolonged exercise in six healthy young men

Time	$\dot{V}_E$	$\dot{V}_A$	$\dot{V}_D$	V_D	V_T	f
7	39 5	31 3	7 2	314	1,920	20 5
30	43 3	34 1	9 2	344	1,885	23 3
60	45 9	35 5	10 4	327	1 698	27 7

18 7 and after 60 min, 18 7 The increase in ventilation, $\dot{V}_E$, thus essentially resulted in an increase in dead space ventilation ($\dot{V}_D$) The $\dot{V}_D$ after 7 min averaged 7 2 l BTPS/min and had risen to 10 4 l BTPS/min after 60 min work The *physiological dead space*, (V_D), averaged 314 ml BTPS after 7 min, 344 ml after 30 min and 327 ml BTPS after 60 min work

The increase in dead space ventilation was thus mainly caused by an increase in respiratory rate The *respiratory quotient*, (RQ), averaged 0 79 at rest, rose to 0 91 after 7 min and then decreased slowly to an average of 0 88 after 60 min work

The *arterial oxygen tension*, (Pa_{O_2}), mm Hg, averaged 91 mm Hg (SD ± 6) at rest. During exercise Pa_{O_2} fell slightly to 88 mm Hg (SD ± 4) after 6 min, and then rose

by Pa_{CO_2}, RQ and V_D

The *pH of arterial blood* was slightly high at rest in all subjects, 7 46 units, which was higher than found earlier in a similar material (Freyschuss and Holmgren 1965) The cause is uncertain If caused by a systematic error, however, the variation during exercise should be true There was a slight initial decrease after 7 min, 7 43 units, and then an increase to 7 45 and 7 47 after 30 and 60 min exercise

The *standard bicarbonate* meq/l, at rest averaged 27 4 meq/l and remained constant 30 min work At 60 min the standard bicarbonate had decreased slightly to 25 meq/l, which is however not significant The simultaneous measurement of pH and standard bicarbonate allows calculation of P_{CO_2} which was one as a check of the high pH values for comparison with the values for P_{CO_2} obtained with the P_{CO_2} electrode The average value at rest was then 39 5 mm Hg, after 7 min 41 8 mm Hg after 30 min 40 8 mm Hg, and after 60 min 36 3 mm Hg There is a discrepancy between the values obtained with the two methods especially after 60 min work, which has been discussed earlier (Holmgren 1964 a) The *rectal temperature* (T) averaged 37 1 a rest 37 3 C after 7 min, 38 0 C after 30 min and 38 1 C after 60 min work

The *diffusing capacity of the lungs for CO* ($D_{L_{CO}}$), was not measured at rest for reasons given above The average $D_{L_{CO}}$ after 7 min work was 35 0 ml min⁻¹ mm Hg⁻¹ (SD ± 5) After 30 min $D_{L_{CO}}$ had increased slightly to 39 2 ml min⁻¹ mm Hg⁻¹ (SD ± 8 7) and after 60 min the average was 37 8 ml min⁻¹ mm Hg⁻¹

TABLE IV Variation of carbon monoxide uptake, $\dot{V}_{CO}$ ml STPD min^{-1}, alveolar carbon monoxide tension, $P_{A_{CO}}$ mm Hg, mean capillary carbon monoxide tension $P_{\bar{c}_{CO}}$, mm Hg and $D_{L_{CO}}$ ml STPD min^{-1} mm Hg^{-1}, in six healthy young men during prolonged non steady state work

Time	$\dot{V}_{CO}$	$P_{A_{CO}}$	$P_{\bar{c}_{CO}}$	$D_{L_{CO}}$
7	7 95	0 2445	0 0148	35 0
30	8 59	0 2410	0 0241	39 2
60	8 54	0 2571	0 0306	37 8

TABLE V Mean values for arterial gas tensions, mm Hg pH, units, and standard bicarbonate mE/l, during prolonged non steady state exercise in six healthy young men (S) and (M) indicate that $P_{a_{CO_2}}$ was determined with a Severinghaus electrode or a microequilibration technique (Andersen *et al* 1960)

Time	$P_{a_{O_2}}$	$P_{a_{CO_2}}$ (S)	$P_{a_{CO_2}}$ (M)	pH	St B
rest	91	39 3	39 5	7 46	27 4
7	88	43 4	41 8	7 43	26 2
30	90	42 2	40 8	7 45	27 0
60	93	41 2	36 3	7 46	25 0

(SD ± 4 3) (8 per cent above the 7 minute value) If the measurements are regarded as triplicate determinations of a constant $D_{L_{CO}}$ the variability in a single determination can be calculated to be 8 4 per cent To analyze the variations of the three parameters in the calculation of $D_{L_{CO}}$, $\dot{V}_{CO}$ $P_{A_{CO}}$ and $P_{\bar{c}_{CO}}$ these will be discussed separately (Table IV) $\dot{V}_{CO}$ averaged 7 95 ml STPD min^{-1} after 7 min (SD 0 92) 8 59 ml STPD min^{-1} after 30 min (SD ± 1 02) and 8 54 ml STPD min^{-1} after 60 min work (SD ± 0 84) These values agree with those that can be predicted from oxygen uptake in a similar material (Freyschuss and Holmgren 1965) The increase in $\dot{V}_{CO}$ is significant and corresponds to the increase in $\dot{V}_{O_2}$. The $P_{A_{CO}}$ averaged 0 2445 mm Hg … there was a significant … 0 0148 mm Hg after 7 … $P_{\bar{c}_{CO}}$ after 30 min and … highest value for COHb was 10 2 per cent

Discussion

The material used in this investigation has been characterized by means of history anthropometric measurement and exercise tolerance studies as being ordinarily physically fit The increase in heart rate during exercise shows that the planned design of the experiment had been attained and that the experimental conditions were similar to those used in the earlier investigation of Ekelund and Holmgren 1964 The work

load performed can be characterized as moderate, in absolute and relative intensity, non-steady-state exercise

The *ventilatory response* during these conditions (Fig 2) was characterized by a marked increase in total ventilation (7.4 l/min). This increase was effectuated by an increase in rate of breathing while tidal volume decreased slightly This type of breathing resulted in an increased dead space ventilation In this study the changes in physiological dead space are less than in those observed in an earlier study (Ekelund and Holmgren 1964), which probably indicates that the V_A/Q_c of the lungs was less affected by the blood shifts that seem to be indicated by the marked increase in heart rate

The P_{aCO_2} decreased only slightly, as did RQ and standard bicarbonate, which indicates that the increase in V_E did not result in an increased alveolar ventilation but only increased ventilation of the dead space

In this material (cf Holmgren 1965 c) there was no difference between electrode and microequilibration technique values at rest During exercise the earlier reported difference was present After 60 min work the difference was marked 4 9 mm Hg The significance of such a difference for the calculation of DL_{CO} and V_A has been discussed in detail by Holmgren (1965) The cause of the difference is at present uncertain and is being investigated The electrode, being the more direct method of measurement and being calibrated directly in mm Hg CO_2, is regarded as the more valid one, and only these data have therefore been used for the calculation of DL_{CO} and V_A

The primary cause of the increase in V_E was either a change in respiratory rate or in V_T A decrease in V_T would cause a decrease in V_A per breath, which would then be compensated by an increase in respiratory rate or vice versa It is not possible from the present data to decide which is the primary change, respiratory rate or depth Gullbring, Holmgren Sjostrand and Strandell (1960) observed an increase in respiratory rate during exercise in sitting position after a 10 per cent decrease in blood volume due to venesection Ekelund and Holmgren (1964) found a similar reaction under identical conditions, but in their study the increased V_D was a result of an increase both in respiratory rate and V_D It seemed possible that orthostatic blood shifts might explain these changes A moderate shift changes the filling of the lung with blood, decreasing the necessary intrathoracic pressure variation for a given V_T, but the capillaries of the lungs are still perfused to such an extent as to keep the alveolar dead space constant as in the present material A marked shift causes an underperfusion of the upper part of the lungs, as in the material of Ekelund and Holmgren (1964), increasing alveolar dead space and V_D

If this mechanism is responsible for the ventilatory reaction in this material one should not expect a decrease in DL_{CO} as there is no indication of overventilated areas which might indicate a decreased capillary blood volume This seems also to be the case DL_{CO} increased 5 units or 12 per cent of the 7-minute value between 7 and 30 min exercise and then decreased slightly Analyses of variance showed that the variation between observations at the different times was not significantly different from the variation between individuals The increase between 7 and 30 min was significant at the 5 per cent level The variability of DL_{CO} measurements using an identical methodological procedure was found by Holmgren (1964 a) to be 7 5 per cent

The present investigation thus shows that DL_{CO} does not change to any major extent during prolonged non steady state exercise The significance level of the increase between 7 and 30 min is only 0 05 If this increase represents a true variation it might imply a successive recruitment of collapsed alveoli The change in the pulmonary blood

volume which can be inferred from the falling central venous and pulmonary artery pressures, the falling stroke volume (Ekelund and Holmgren 1964) and the decreasing heart volume (Ekelund, Holmgren and Ovenfors 1964), does not seem to affect the perfusion of the lung or the number of open capillaries in the present material

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Studies on the Elimination of Exogenous Lipids from the Blood Stream

The effect of fasting and surgical trauma in man on the elimination rate of a fat emulsion injected intravenously

By

DAG HALLBERG

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Abstract

Hallberg D *Studies on the elimination of exogenous lipids from the blood stream The effect of fasting and surgical trauma in man on the elimination rate of a fat emulsion injected intravenously* Acta physiol scand 1965 65 153—163 Single i v injections of a fat emulsion were given to 2 groups of

the surgery group The fractional removal rate operating at low concentration of the fat emulsion in plasma was unchanged in the control group and increased in the surgery group Observations on recirculating lipid fractions are reported The results are discussed with regard to factors influencing the two rate constants

The kinetics for the elimination of intravenously injected fat emulsion from the blood stream in dog and man has been described earlier (Carlson and Hallberg 1963 Hallberg 1964) In both species it can be described by a maximal elimination capacity above a certain triglyceride (TG) concentration and a fractional removal rate below It was also found that inter as well as intra individual differences in the size of the rate constants could occur The intra individual differences were found in the maximal removal capacity while the fractional removal rate remained essentially unchanged in repeated tests in subjects fasting overnight Hallberg 1965 b

TABLE I Sex, age, weight, height, diagnosis, and surgical treatment of patients studied at

Non surgery

No	Sex	Age years	Weight kg	Height cm	Diagnosis
1	F	62	65	167	Fracture of femur (convalescent)
2	F	57	42	160	Fracture of femur (convalescent)
3	M	28	80	187	Nephrolithiasis (free of symptoms)
4	M	32	65	174	Fracture of femur (convalescent)
5	M	40	67	172	Nephrolithiasis
Mean		44	63	172	

M = Male, F = Female

The nutritional state is considered not to affect the removal rate of chylomicrons from the blood stream in rats (Bragdon, Havel, Gordon 1957, Fredrickson, McCollester, Ono 1958, Bragdon and Gordon 1958, Olivecrona *et al* 1961) but is has a profound influence upon the tissue distribution and rate of oxidation of chylomicrons (Bragdon and Gordon 1958, Morris 1958, Fredrickson *et al* 1958, cf Robinson and French 1960, cf Olivecrona 1962)

The nutritional state, however, also influences the plasma TG concentration in a complex manner Increase in plasma TG are observed after starvation (Carlson and Wadstrom 1956) and after periods of high-carbohydrate dietary intake (Waterhouse *et al* 1964) *Decreases are observed after acute carbohydrate feeding* (Havel 1957, Bragdon *et al* 1957) The turnover rate of plasma TG is influenced by the nutritional state in rats (Laurell 1959)

The i v injection of chylomicrons is followed by recirculation of the chylomicron TG in other plasma lipoproteins (cf Robinson and French 1960, cf Olson and Vester 1960 cf Dole and Hamlin 1962 cf Olivecrona 1962) The i v injection of artificial fat emulsion alters endogenous plasma TG concentration in man (Hallberg 1965, a b)

Surgical trauma in man may cause changes in lipid metabolism These changes are not fully understood, but one of the main features is an increased demand and expenditure of calories (cf Moore 1960) Indices of metabolic changes are decreased glyceride level and increased free fatty acid level in plasma after abdominal surgery in man (Wadström 1959)

Parenteral nutriments are sometimes given to patients after abdominal surgery when they cannot take food by mouth The present investigation was designed to study the elimination from the blood stream of an artificial fat emulsion before and after abdominal surgery in man Patients with a comparable period of fasting without abdominal surgery served as controls

The results showed that the maximal elimination capacity of fat emulsion from the blood stream increased during the fasting state and that abdominal surgery accentuated this phenomenon

different nutritional states with and without abdominal surgery

Surgery						
No	Sex	Age years	Weight kg	Height cm	Diagnosis	Surgical treatment
6	M	43	84	180	Duodenal haemangioma	Electrocoagulation
7	M	36	67	169	Incisional hernia	Repair
8	F	47	64	153	Cholelithiasis	Cholecystectomy
9	F	44	60	163	Cholelithiasis	Cholecystectomy
10	F	66	54	172	Colostomy (post volvulus)	Closure of colostomy
11	F	55	69	166	Cancer of colon	Partial colectomy
Mean		49	66	167		

Material and methods

Subjects All subjects were patients admitted to the surgical department because of disorders requiring surgery They were all in good condition at the time of the investigation, and no complications were noted during their stay in hospital Age, weight, height, sex, diagnosis, and surgical management are given in Table I All patients could move around in the ward at the time of the first test No restriction in activity was given to the unoperated subjects between the two tests (see below)

Procedure After an overnight fast (15 hrs) a single i. v. injection of an artificial fat emulsion (Intralipid® 20 %, VITRUM, Stockholm) was given and blood samples were taken for TG analyses The subjects were then not allowed to eat food or take calories until after another test which was performed the next morning (39 hrs of fasting) Pulse rate, blood pressure (cuff manometer) and body temperature were recorded before each test Abdominal surgery (see Table I) was performed after the first test in 6 of 11 subjects In the operated cases anesthesia was induced with Narkotal®, ASTRA (5-(2 bromallyl)-N methyl 5 isopropyl malonyl-carbamide) and continued with nitrous oxide, oxygen, and the muscle relaxing agent Celocurin®, VITRUM (succinylcholin) under controlled respiration. No blood transfusions were given The subjects in the surgery group were only given 2,000 ml of saline slowly i. v. during the day of operation The subjects in the non-surgery group were allowed to drink water and coffee Oxicon® ACO ((-) 14-hydroxi 3-metoxi N-metyl-6-oxo-4,5-epoximorfinan) was given to the surgery group and to one of the unoperated subjects (no 3) to control pain during the time between the two tests

The fat emulsion was injected i. v. into a cubital vein within 2 min The amounts of fat injected varied between 0 30 and 0 50 g kg and are set out in Table IV Blood samples for analyses were taken from a permanent needle in the other arm All samples were collected in heparinized tubes and stored *in ice water not longer than 4 hrs before processing* About 7 ml of blood were taken each time

Analysis The blood samples were centrifuged at slow speed and plasma was taken for TG analyses before and after separation into three fractions in a polyvinylpyrrolidone (PVP) density gradient according to Gordis (1962) method as described earlier (Hallberg 1964)

The gradient separates fat emulsion to the top in the gradient tube (top fraction and other plasma TG into two fractions which remain in the lower part of the gradient tube (middle and bottom fraction) (Hallberg 1964)

The TG analyses were performed by Carlson's (1963) method Triplicate determinations were made on each extract The preinjection plasma samples were extracted in duplicate The errors of the analysis have been given earlier (Hallberg 1964) The mean recovery after gradient

TABLE II Pulse rate, blood pressure, body temperature and triglyceride (TG) concentration

Non surgery				
Case No	Pulse rate per min	Blood pressure mm Hg	Body temp C°	Fasting TG mmole/L
1	82–80	150–150	37 2–36 8	0 68–0 61
2	75–81	135–130	37 2–37 2	1 29–1 36
3	82–84	125–130	36 8–36 6	1 46–1 06
4	86–86	120–120	36 7–36 8	1 39–1 63
5	72–80	130–130	36 8–36 8	1 10–1 38
Mean	79–83	132–132	36 9–36 8	1 18–1 22
d	3 4	0	–0 1	0 03
	±2 2	±0	±0 1	±0 13

The first figures in each column are values after 15 hrs of fasting and the second figures after ... icance of the differenced (d=39 hrs—15 hrs) * =P<0 01 *** =P<0 001

separation in this study was 95 % ± 1 0 SEM (n = 289) Analyses of TG on whole plasma and its gradient fractions were performed on different days The recovery in each series of samples was always of the same magnitude Gradients with a recovery outside 2×SD were excluded from calculations

Calculations The TG concentrations in plasma and its fractions at different times were plotted on both linear and semilog graphs The rates of elimination of the fat emulsion (top fraction in the gradient) from the blood stream were determined from the slopes of the curves (Hallberg 1965 a, 1965 b) The critical concentration (C mmole/l) e g the concentration at which the maximal elimination capacity changes to a fractional removal rate, was determined from the formula $K_1 - K_2$ C where K_1 is the maximal removal capacity (mmole/l min) and K_2 is the fractional removal rate (per min) When the curve showed only an exponential elimination rate, a maximal elimination capacity was considered to be present but to be greater than the capacity at its highest concentration Such figures are given within parentheses in Table IV Statistical methods used were as described by Snedecor (1957)

Results

The 2 groups of subjects with and without surgery differed with regard to diagnosis They were comparable with regard to mean age, mean b w and mean height (Table I)

After 15 hrs of fasting there was no significant difference between the groups with regard to mean blood pressure mean pulse-rate mean body temperature and mean TG concentration in plasma (Table II After 39 hrs of fasting the groups were not comparable with regard to the last mentioned parameters These did not change in the unoperated group during the fasting period in the operated group there was a significant increase in pulse-rate (P 0 01) and body temperature (P 0 001 In all the 6 operated cases the TG concentration decreased while in 3 of the 5 controls it increased (Table II) Separation of plasma in the density gradient into fractions showed no significant changes either during the fasting period or between the 2 groups Table III)

in plasma after 15 and 39 hrs of fasting in subjects without and with abdominal surgery

Surgery

Case No	Pulse rate per min	Blood pressure mm Hg	Body temp C°	Fasting TG mmole/L
6	68—96	130—135	36 8—37.8	1.37—1 25
7	88—100	130—145	36 6—38 0	1 97—1 95
8	72—94	135—130	37 1—37 8	0 93—0 93
9	68—74	120—135	37 0—37 8	0 78—0 73
10	80—100	110—120	37 2—37 8	0 93—0 71
11	98—112	160—140	36 9—38 0	1 60—0 84
Mean	79—96	131—134	36 9—37 9	1.27—1 07
d	17**	3	1 0***	—0 20
	±5 2	±5 7	±0 1	±0 12

39 hrs of fasting ± indicates standard error of mean ** and ** indicate the degree of signif

Table III Mean triglyceride (TG) concentration and standard error of mean before and after separation into three fractions (top, middle, and bottom) in the density gradient in plasma from subjects without and with abdominal surgery after different hours of fasting

	I		II	
	15 hrs	39 hrs	15 hrs	39 hrs
Plasma TG	1 18	1 22	1 27	1 09
	± 0 13	± 0 18	± 0 19	± 0 19
Top	0 04	0 08	0 01	0 03
	± 0 001	± 0 04	± 0 001	± 0 001
Middle	0 49	0 59	0 41	0 34
	± 0 10	± 0 15	± 0 12	± 0 11
Bottom	0 71	0 83	0 69	0 64
	± 0 07	± 0 17	± 0 14	± 0 13
Σ	1 24	1 50	1 11	1 01
Recovery %	105	123	88	93

I = Non-surgery group (n = 5) II = Surgery group (n = 6)

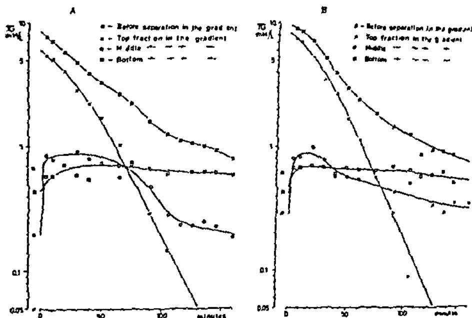


Fig 1 Triglyceride concentration in plasma before and after separation into three fractions in density gradients Samples taken before and after intravenous injection of fat emulsion in case No 1 (0 4 g fat/kg body weight) Semilogarithmic scale A. After 15 hours of fasting B After 39 hours of fasting

Elimination of injected fat emulsion

The injection of fat emulsion increased the total plasma TG concentration This increase was followed by a decrease In the unoperated subjects no marked differences were observed between the tests performed after 15 and 39 hrs of fasting The operated group showed a faster decrease at the 39 hr test All curves for the total TG concentration were complex and difficult to interpret (Fig 1) Each fraction in the density gradient will be presented separately

Top fraction This fraction contains the emulsion TG The elimination curves obtained were interpreted as either single exponential or composed of a linear part at high TG concentration and exponential at low TG concentration The rate constants for the elimination of the fat emulsion from the blood stream are given in Table IV After 15 hrs of fasting there were no significant differences in these constants between the two groups After 39 hrs of fasting both K_1 and K_2 were greater in the operated group

In the unoperated subjects the mean maximal elimination capacity (K_1) after 39 hrs of fasting was increased by 50 per cent ($P < 0.05$) No differences were observed in the fractional removal rates K_2 (Table IV

In the operated subjects the mean maximal elimination capacity (K_1) after 39 hrs of fasting was increased by > 250 per cent ($P < 0.05$) (Fig 2 The mean fractional removal rate (K_2) increased from 0 05 to 0 09 Case No 10 was excluded from the mean calculations because of the very rapid elimination rate after 39 hrs of fasting too few samples were obtained for accurate determination of the rate constants In 2 subjects

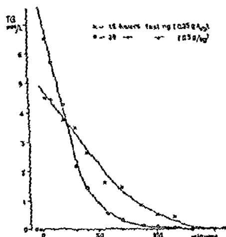


Fig 2 Triglyceride concentration in the top fraction after density gradient separation of plasma Samples taken at intervals after intravenous injection of fat emulsion in case No. 11 Tests performed after 15 hours of fasting (0 35 g fat/kg) and 39 hours of fasting (after surgery, 0 5 g/kg)

(no 8 and 9) the maximal elimination rates were calculated from the highest value of the single exponential curves, and were thus probably too small

Middle fraction This fraction contains a part of the plasma TG After the injection of the fat emulsion, the general shape of the curve for this fraction consisted of an initial rise, sometimes up to a plateau (see Fig 1), which was followed by a decrease Inter individual variations in the curves were apparent. In both groups the test after 39 hrs of fasting gave values that were lower (not significantly) than after the 15-hr test The declining part of the curve was steeper after prolonged fasting in 8 of 11 cases (Fig 1) The duration of the plateau when present was always shorter after the longer fasting period (Fig 1) This was a striking observation in the surgery group Owing to the considerable variations, the number of subjects in each group was too small to demonstrate any significant differences, if present, either within each group or between the groups

Bottom fraction This fraction contains endogenous plasma TG After the injection of the fat emulsion the TG concentration increased more slowly than in the middle fraction (Fig 1) Inter-individual variations were not so obvious as in the middle fraction The general pattern of the curves showed that after 39 hrs of fasting the increases were larger than after 15 hrs in the non surgery group and the reverse in the surgery group In view of the considerable variations the number of subjects in each group was too small to demonstrate any significant differences if present, either within each group or between the groups

Discussion

The kinetic behaviour of TG in plasma after the injection of a fat emulsion (Intralipid®) has been described earlier During disappearance of the fat emulsion from the blood stream other fractions of plasma TG changes in concentrations (Hallberg 1964) These changes were followed after fractionation of plasma in a density gradient by a modification of Gordis technique (cf Hallberg 1965 a, b) Inter- as well as intra indi

TABLE IV. Rate constants for the elimination from the blood stream of intravenously injected

Non-surgery

Patient No	State: Hours of fasting	Surgery	Injected dose g/kg	K_1 mmole/L/min	K_2 min^{-1}	$C = \frac{K_1}{K_2}$ mmole/L
1	15 h	–	0 40	0 09	0 05	1 8
	39 h	–	0 40	0 13	0 05	2 6
2	15 h	–	0 40	0 07	0 04	1.75
	39 h	–	0 40	0 07	0 04	1 75
3	15 h	–	0 40	0 05	0 03	1 67
	39 h	–	0 40	0 09	0 03	3 00
4	15 h	–	0 40	0 06	0 03	2 00
	39 h	–	0 40	0 09	0 03	3 00
5	15 h	–	0 35	0 03	0 04	0 75
	39 h	–	0 35	0 08	0 04	2 00
Mean	15 h	–		0 06	0 04	1 5
	39 h	–		0 09[1]	0 04	2 3

(I) Excluded from all mean values (see text) [1] Indicates the degree of significance of the elimination rate

vidual variations in the curves were observed (Hallberg 1965 b) These observations were confirmed in this study

Few facts are known about factors determining the size of the rate constants (K_1 and K_2) If the elimination of the fat emulsion is considered to take place in one organ (the liver ?) then the maximal elimination capacity (K_1) must contain at least one "*organ factor*" The magnitude of circulation ("*flow-factor*") through such an organ may influence the fractional removal rate (K_2) An increased flow results theoretically in a decreased half-life of a test substance in the circulation, provided that the amount eliminated is below the maximal elimination capacity of the organ and the non flow-dependent factor is independent of the flow-factor"

In this study it was found that the maximal elimination capacity (K_1 from the blood stream of fat emulsion was influenced by the nutritional state Below the "critical concentration" the elimination rate K_2 was always *independent of the nutritional state* in the non-surgery group This last observation is in agreement with earlier observations with chylomicrons (Fredrickson *et al*, Bragdon and Gordon, Olivecrona It suggests that earlier studies were performed below the critical concentration The subjects

fat emulsion at different hours (h) of fasting in subjects without and with abdominal surgery

Surgery

Patient No	State: Hours of fasting	State: Surgery	Injected dose g/kg	K_1 mmole/L/min	K_2 min^{-1}	$C = \frac{K_1}{K_2}$ mmole/L
6	15 h	−	0.40	0.03	0.03	1.00
	39 h	+	0.40	0.08	0.03	2.67
7	15 h	−	0.40	0.04	—	—
	39 h	+	0.40	0.10	0.05	2.00
8	15 h	−	0.30	0.11	0.05	2.40
	39 h	+	0.30	(>0.34)	0.23	(>2.30)
9	15 h	−	0.30	0.08	0.08	1.00
	39 h	+	0.30	(>0.20)	0.08	(>2.50)
10 (1)	15 h	−	0.40	(>0.24)	0.06	(>4.00)
	39 h	+	0.40	(>0.48)	0.12	(>4.00)
11	15 h	−	0.35	0.06	0.03	2.00
	39 h	+	0.50	0.13	0.05	2.60
Mean	15 h	−		0.06	0.05	1.2
	39 h	+		(>0.17)*	0.09	(>1.9)

difference (39 hrs — 15 hrs) ($P<0.05$) K_1 = Maximal elimination capacity K_2 = Fractional

in the surgery group had both increased maximal elimination capacity (K_1) and an increased fractional removal rate (K_2)

The nutritional state is thus one factor (possibly "organ factor") influencing the maximal elimination capacity (K_1) in the non-surgery group If the 2 groups were comparable with regard to the "organ factor", the 39 hr fasting state in the surgery group can only *partly* explain the three fold increase in maximal elimination capacity The surgery patients had an increased fractional removal rate of the top fraction TG (K_2 from 0.05—0.09) They also had a significantly increased pulse-rate and body temperature and unchanged blood pressure after 39 hrs of fasting These facts may be taken as an index of increased circulation and possibly also of an increased perfusion through the eliminating organs In the non-surgery group these circulatory factors were unchanged and the fractional removal rate did not in fact, change during the fasting period

The differences between the mean values for the changes in middle fraction TG concentration at comparable times after the injection of the fat emulsion and during different nutritional states, were too small to be statistically significant It was not possible to

compare the rate constants governing the middle fraction TG concentration The trend suggests either a smaller amount of recirculating TG or an increased turnover rate after prolonged fasting The changes induced by the fat load were similar in both groups, but the changes in middle fraction TG concentration in the surgery group seem to be less pronounced The curves for the middle fraction in Fig 1 suggests a rise up to a level This suggests a constant infusion into this plasma pool Such a constant infusion was earlier suggested to emanate from the linear elimination of the fat emulsion (the top fraction) from the plasma pool (Hallberg 1965 b) The duration of the plateau of the curve for the middle fraction was always of about the same duration as the linear part of the curve for the top fraction (Fig 1A)

The differences between the mean values for the changes in bottom fraction TG concentration, at comparable times after the injection of the fat emulsion and during different nutritional states, were too small to be significant Assuming that the rate constants (not determinable in this study), governing the 39 hr curves from the tests in the non surgery group, demonstrate the normal size (judged from the TG level) during a fat load after prolonged fasting, the rate constants in the surgery group will be greater but not to such an extent as to give a significant change in the bottom fraction TG level [illegible] non surgery group and lower [illegible]

group Wadstrom's (1959) finding that plasma TG decreased after abdominal surgery can be taken as an index of the same mechanisms A decreased postoperative plasma TG was also observed in this study

Increased circulation after trauma concomitant with changes in the nutritional state may kinetically explain both the decreased fasting TG in plasma and the observed changes in the three plasma fractions after the fat load to the surgery patients

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Acta physiol. scand. 1965. 65. 164—175

From the Department of Physiology, University of Göteborg, Sweden

The Location of the Thalamic Relay in the Spino-Cervico-Lemniscal Path

By

S. LANDGREN, A. NORDWALL and C. WENGSTRÖM

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Abstract

Landgren, S., A. Nordwall and C. Wengström. *The location of the thalamic relay in the spino-cervico-lemniscal path.* Acta physiol. scand. 1965. *65.* 164—175. — Cats anesthetized with nembutal were used. The dorsal half of the lateral funicle and the dorsal columns at L1 as well as the superficial radial nerve were dissected. The cerebellum was removed. Focal potentials and action potentials of single units were recorded in the contralateral thalamus in response to electrical stimulation of the three inputs. Ipsilateral hemisection rostral to C1 did not abolish the response to stimulation of the dorsolateral funicle. It was therefore concluded that this response was evoked via the spino-cervico-lemniscal path. It was shown that the thalamic relay of this path was located in a border zone within *n. centralis postero-lateralis.* This zone covered the rostral pole, the dorsal, lateral and ventro-lateral aspect of the nucleus and surrounded like a shell the somatotopically organized hindlimb region dominated by the dorsal column response. Convergence between the spino-cervico-lemniscal, the dorsal-column-lemniscal and the superficial radial paths was a characteristic feature of the border zone. In a population of 62 thalamic units, isolated in the zone of convergence, 20 units were discharged only via the spino-cervico-lemniscal path. Out of the remaining units 31 were discharged by two or by all three of the stimulated paths.

The experiments leading up to the definition of the spino-cervical tract and its continuation via Morin's path (Morin 1955) to the cerebral cortex were recently reviewed by Lundberg (1964). Low threshold cutaneous afferents contribute to this path. They are monosynaptically relayed in the dorsal horn of the spinal cord, and the secondary axons ascend dorsomedially in the ipsilateral lateral funicle. The second synapse is found in *n. cervicalis lateralis* of Rexed and Brodal (1951). The path then crosses the midline at the level of the first and second cervical segment and ascends with the medial lemniscus to the thalamus. Its projection to the cerebral cortex was studied by Norsell and Voorhoeve (1962) who found that impulses transmitted via the spino-cervico-lemniscal path reached the cortex earlier than those mediated via the dorsal-column lemniscal path.

Lundberg (1964) described the location of the neurones in the dorsal horn giving rise to the axons of the spino-cervical tract. The afferent inflow to single neurones in *n. cervicalis lateralis* was studied by Gordon and Jukes (1963) and by Oswaldo-Cruz and Kidd (1964). In addition Gordon and Jukes (1963) studied their ascending projections by

antidromic stimulation of the axons in the medial lemniscus. A detailed study of the spino-cervico-lemniscal inflow to the neurones in the second somatic sensory area of the cortex was presented by Andersson (1962). The thalamic synaps of this path has so far attracted less interest. Observations of degenerative processes in *n. cervicalis lateralis* following lesions in the lateral part of midbrain tegmentum led Morin and Catalano (1955) and Catalano and Lamarche (1957) to the conclusion that the axons from this nucleus ascended in the medial lemniscus. Morin and Thomas (1955) also demonstrated a degeneration in the dorsolateral part of the opposite medial lemniscus after lesions in *n. cervicalis lateralis* and therefore concluded that the path ended laterally in the ventro-lateral thalamic nucleus. Busch (1961) and Grant and Boivie (1965, personal communication) made lesions in n. cervicalis lateralis and were able to follow the degeneration into the lateral part of the ventrobasal complex. This report will present evidence concerning the location of the thalamic relays in a border zone of *n. ventralis posterolateralis* enclosing the somato-topically organized part of this nucleus. Evidence of convergence between the spino-cervico-lemniscal and the dorsal-column lemniscal paths in this border zone will also be presented. A preliminary report of these findings was published (cf. Landgren, Nordwall and Wengstrom, 1965).

Methods

Cats anesthetized with nembutal were used for the experiments. The dorsal columns and the dorsal half of the lateral funicle were dissected at the level of the first lumbar segment and prepared for electrical stimulation. The superficial radial nerve was also dissected for stimulation. The cerbellum was removed. The skull was opened over the cerebral hemisphere contralateral to the stimulated paths to admit the recording electrode penetrations.

Focal potentials and extracellular action potentials from single units were recorded in the thalamus with glass pipette microelectrodes filled with 4 M NaCl. Systematically placed transversal grids of tracks were made through the thalamus with the aid of the Horsley Clarke technique. Only one microelectrode was used in each grid. Prussian blue reference spots placed with steel microelectrodes were used for dorso-ventral localisation. The course of the tracks were identified on histological 40µ thick serial sections stained with toludin blue or luxol fast blue (cf. Kluver and Barrera, 1953). The observations were localized on reconstructions made from the histological sections. A detailed description of the recording and localizing technique was given by Eisenman, Landgren and Novin (1963). The nomenclature of the thalamic nuclei used in this report was adopted from the atlas of Jasper and Ajmone-Marsan (1954).

Results

1. *The thalamic response to electrical stimulation of the spino-cervical tract*

Electrical stimulation of the dorso-lateral fascicle of the spinal cord at the level of L1 evokes a focal potential in *nucleus ventralis posterolateralis* (VPL) of the thalamus. As shown in Fig. 1 A, the focal potential consists of a negative wave with a latency of 5 msec (range in different experiments 4.0—6.3 msec) and a duration of approximately 10 msec. Action potentials of single cells appear on the crest and falling phase of this wave and it is therefore considered to be of postsynaptic origin. When recorded from the site of its maximum amplitude, it is often preceded by a positive wave of short duration, which is interpreted as the initial phase of a positive negative presynaptic spike. The negative phase of this spike may sometimes be seen as a notch on the rising phase of the postsynaptic potential.

The threshold of the thalamic focal potential is similar to that of the compound action potential recorded from *n. radialis superficialis* in response to electrical stimulation. When

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The experiments leading up to the definition of the spino-cervical tract and its continuation via Morin s path (Morin 1955) to the cerebral cortex were recently reviewed by Lundberg (1964 Low threshold cutaneous afferents contribute to this path. They are monosynaptically relayed in the dorsal horn of the spinal cord, and the secondary axons ascend dorsomedially in the ipsilateral lateral funicle The second synapse is found in *n cervicalis lateralis* of Rexed and Brodal (1951) The path then crosses the midline at the level of the first and second cervical segment and ascends with the medial lemniscus to the thalamus Its projection to the cerebral cortex was studied by Norsell and Voorhoeve (1962 who found that impulses transmitted via the spino-cervico-lemniscal path reached the cortex earlier than those mediated via the dorsal-column lemniscal path.

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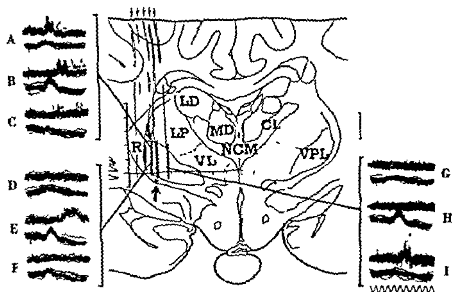


Fig 3 Outline drawing of the histological section shown in the photomicrograph of Fig 4 The traces left by five parallel microelectrode tracks in adjacent serial sections are superimposed on the diagram Prussian blue reference points for dorso-ventral localization are indicated by arrows The heavy bars along the four medial tracks indicate the responding region. The groups of records show focal potentials evoked by stimulation of the spino-cervical tract (A D G) the dorsal columns (B E H) and the superficial radial nerve (C F I) The lower record in each pair was obtained from the dorsal surface of the medulla oblongata The recording sites of the upper records are indicated on the diagram A—C dorso-laterally D—F centro-laterally and G—I centro-medially in VPL Time 2 msec Voltage calibration 0 5 mV

Abbreviations used for indication of the thalamic nuclei in this and the subsequent diagrams

Cd	n caudatus	NR	n. ruber
CL	n centralis lateralis	Pul	Pulvinar
CM	n centrum medianum	R	n reticularis
GL	corpus geniculatum laterale	THP	tractus habenulo-peduncularis
GM	corpus geniculatum mediale	TO	tractus opticus
LD	n lateralis dorsalis	VL	n. ventralis lateralis
LP	n lateralis posterior	VPL	n. ventralis postero-lateralis
MD	n medialis dorsalis	VPM	n ventralis postero-medialis
NCM	n centralis medialis		

features are in agreement with those of the pathway described by Lundberg and Oscarsson (1961) The focal potentials evoked in VPL by stimulation of the dorsolateral fascicle at L1 are thus interpreted as elicited by the spino-cervico-lemniscal path.

2 *The location of the thalamic responses*

The thalamic responses evoked by electrical stimulation of the contralateral spino-cervical tract at L1 the dorsal columns at L1, and the contralateral superficial radial nerve were studied in 244 microelectrode penetrations The penetrations were systematically arranged in transversal grids of parallel tracks distributed throughout the rosto-caudal extent of the ventrobasal complex The course of the penetrations was



Fig 4 Photomicrograph of a histological transversal section through the diencephalon of the cat. The section was selected from the level where the grid of recording microelectrode tracks passed VPL Faint traces left by the tracks are indicated by the arrows

localized on histological serial sections 54 reconstruction diagrams of the type shown in Fig 3 were made as described in methods

The results of a typical experiment are shown in Fig 3 A transversal grid of five tracks was made with the same microelectrode and with a distance of 0 5 mm between each track The tracks went approximately parallel to the sagittal plane and passed VPL and *n reticularis* (R) in the section from which the photomicrograph of Fig 4 was made The diagram of Fig 3 is an outline drawing of the section Responses to one of the above mentioned inputs or from some points to all of them were recorded along those parts of the four medial tracks marked by a heavy bar in the diagram of Fig 3 There is a good agreement between the extent of the responding region and the border lines of VPL drawn from the histological section The track passing R lateral to VPL gave negative results

In order to illustrate the typical responses recorded along the penetrations of Fig 3 three recording sites within VPL were selected The upper record in each pair shown in Fig 3 A—I was obtained in these sites The spino-cervico lemniscal path evoked a focal potential of short latency in the dorso-lateral recording site (A) This path however was not the only input to this region The dorsal-column lemniscal path from the hindlimb as well as the cutaneous path from the forelimb also evoked responses there (B and C) The spino-cervico-lemniscal response was generally largest in amplitude but the response from the forelimb was not necessarily the smallest

A different picture was obtained from the recording site located centro-laterally in VPL At this point a large focal potential was evoked by the dorsal-column lemniscal path (E) but the other paths were ineffective (D F) Centro-medially the response to the superficial radial nerve was large (I) but no response was evoked by the other two paths (G H)

The location of the centro-lateral and centro-medial recording sites is in full agreement with the description of the somatotopical organization of VPL given by Mountcastle and Hennemann (1949) and by Rose and Mountcastle (1952) Convergence between forelimb and hindlimb paths within VPL is however not previously described The spatial extent of the region showing convergence and its relation to the somatotopically organized part of VPL is illustrated in Fig 5 The 4 diagrams are based upon results obtained in 4 typical grids of penetrations selected according to their rostro-

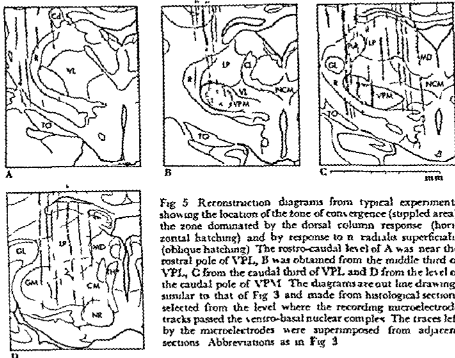


Fig 5 Reconstruction diagrams from typical experiments showing the location of the zone of convergence (stippled area) the zone dominated by the dorsal column response (horizontal hatching) and by response to n radialis superficialis (oblique hatching) The rostro-caudal level of A was near the rostral pole of VPL, B was obtained from the middle third of VPL, C from the caudal third of VPL and D from the level of the caudal pole of VPM The diagrams are out line drawings similar to that of Fig 3 and made from histological sections selected from the level where the recording microelectrode tracks passed the ventro-basal nuclear complex The traces left by the microelectrodes were superimposed from adjacent sections Abbreviations as in Fig 3

caudal localization in VPL They are in agreement with the results of other grids located in similar positions or in between those selected for illustration. The grid of Fig 5 A passed through the rostral third of VPL near the rostral pole of the nucleus B was located in the middle third of VPL 200μ rostral to the rostral pole of *corpus geniculatum laterale* (GL) C in the caudal third of VPL 200μ caudal to the rostral pole of GL and D in the region of the caudal pole of *nucleus ventralis posteromedialis* (VPM)

The stippled areas in the diagrams show the region with a short latency response to stimulation of the spino-cervical tract at L1 and in addition a response to stimulation of the dorsal columns at L1 as well as to stimulation of the contralateral superficial radial nerve This region will henceforth be called the *zone of convergence* It forms a border region within VPL and encapsulates like a shell the rostral pole the dorsal lateral and ventro-lateral aspect of the nucleus It encloses partly the somatotopically organized hindlimb region dominated by the dorsal-column lemniscal response and indicated in the diagram by the horizontal hatching The oblique hatching indicates the location of the region dominated by the forelimb response.

As shown in Fig 5 D responses to all the three inputs were recorded near the caudal pole of VPM where the medial lemniscus enters the ventrobasal complex The focal potentials recorded here were however small in amplitude and it is uncertain whether they were pre or postsynaptic in origin The posterior group of nuclei investigated by Poggio and Mountcastle (1960 corresponds to a region near the caudal pole of VPL and VPM The group includes the anterior part of *corpus geniculatum mediale* GM found

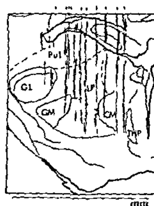


Fig 6



Fig 7.

Fig 6 Reconstruction diagram obtained from a recording grid through the posterior group of nuclei of Poggio and Mountcastle (1960) Responses were evoked from the spino-cervical tract, the dorsal columns and the superficial radial nerve within the stippled area The vertical hatching indicates a region responding to the two last mentioned inputs but not to the spino-cervical tract Abbreviations as in Fig 3

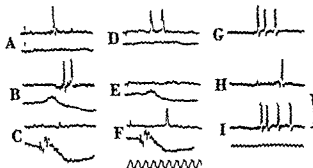
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at levels between diagram C and D of Fig 5 The results of an experiment with penetrations at such a level are shown in Fig 6 The vertical hatching, located in the anterior part of GM indicates a region within which focal potentials were evoked by stimulation of the dorsal columns and the superficial radial nerve Poggio and Mountcastle (1960) described convergence between cutaneous paths from the forelimbs and hindlimbs onto *neurones in this region, and our findings are compatible with their observations* No response was, however, evoked here by stimulation of the spino-cervical tract, but the more medially located stippled area received convergence from all the three inputs The experiment shows that certain parts included in the posterior group of nuclei do not receive an inflow from the spino-cervico-lemniscal path

The zone of convergence is located in VPL. The stippled area in the diagrams B and C does, however, trespass the borderlines between VPL, *n lateralis posterior* (LP), and R These morpho- [illegible]

tion of the dorsal columns or the radial nerve

Exceptions from the above described pattern of convergence between the three inputs were occasionally observed in the zone of convergence Thus, in one experiment a recording site was found dorsolaterally in VPL from which a focal potential was recorded only in response to stimulation of the spino-cervico-lemniscal path In the caudal third of VPL, where the forelimb relays occupy a larger part of the nucleus than the relays of the dorsal column path from hindlimb (cf Fig 5 C), the focal potential evoked in the zone of convergence by stimulation of the superficial radial nerve was often larger in amplitude than that evoked via the dorsal-column-lemniscal path



[illegible]

calibration 1 mV

3 *The difference in latency between the dorsal-column-lemniscal response recorded in the zone of convergence and that recorded in the somatotopically organized part of VPL*

As mentioned above the latency of the thalamic response evoked via the spino-cervico-lemniscal path was shorter than that of the response to dorsal-column stimulation The latency of the latter was however, about 1 msec shorter when recorded from the somatotopically organized centro-lateral part of VPL than it was when recorded from the zone of convergence These latency differences are illustrated in Fig 7 Similar differences were found in all our experiments The latency of the postsynaptic component of the focal potential evoked via the dorsal-column lemniscal path in the somatotopically organized part of VPL was thus generally between 6 and 7 msec and the same latency in the zone of convergence was 7—9 msec

4 *Evidence of convergence obtained by single unit analysis*

Action potentials were recorded extracellularly from 109 single units located in VPL Out of these, 62 units were located in the zone of convergence 17 in the region dominated by the dorsal-column focal potential and 30 in the region dominated by the focal potential evoked by the superficial radial nerve.

Units responding only to one of the three inputs were seen in 50 per cent of the population collected in the zone of convergence The other 50 per cent showed various patterns of convergence Three of the most common ones are seen in Fig 8 The upper records of the three columns A—C D—F and G—I were obtained from three different units The unit of A—C was discharged by the spino-cervico-lemniscal and the dorsal-column lemniscal paths that of D—F by the spino-cervico-lemniscal and the superficial radial paths and finally that of G—I by all three inputs.

Further details about the observed types of afferent inputs may be found in Table I It may be seen in column 3 that as many as 20 out of 62 units found in the zone of convergence responded only to stimulation of the spino-cervical tract Units discharged

TABLE I Patterns of convergence observed on 109 VPL cells

Location in VPL	Type of convergence	Number of cells	Stimulus	Range of latency to 1st spike, msec
Zone of convergence	SC alone	20	SC	4.7–11.5
	DC alone	5	DC	10.5–15.7
	SR alone	6	SR	5.0–8.4
	SC+DC	8	SC	4.0–10.0
			DC	8.0–15.0
	SC+SR	11	SC	4.5–10.0
			SR	6.4–14.0
	SC+DC+SR	10	SC	5.2–12.4
			DC	6.8–16.7
			SR	6.5–11.0
	DC+SR	2	DC	10.0
			SR	13.0
Dorsal column zone	DC alone	15	DC	7.2–10.6
	SC alone	1		
	SC+DC	1		
Radial zone	SR alone	30	SR	6.6–7.2

SC = Spino-cervico-lemniscal path
DC = Dorsal- column-lemniscal path from contralateral hindlimb
SR = Superficial radial path from contralateral forelimb

only via the dorsal-column-lemniscal path or only via the superficial radial path were also observed.

As would be expected, the great majority of the units found in the somatotopically organized parts of the nucleus were discharged by one input only. In the dorsal column zone 15 units out of 17 were discharged via the dorsal-column lemniscal path. One unit in this group was, however, discharged via the spino-cervico-lemniscal path and one showed convergence between this and the dorsal column-lemniscal paths. All units located in the radial zone responded only to the superficial radial nerve.

The latencies given in the fifth column of Table I to the first spike in the response evoked by electrical stimulation are the shortest ones observed. The stimulus used was supramaximal in strength as judged from the amplitude of the thalamic focal potential. The range is that found within the observed group of units. The latencies of the responses to the spino-cervico-lemniscal and the dorsal-column lemniscal paths may be of greatest interest as these two paths should have the same length. There is good agreement between the latencies of the focal potentials and the single unit responses. Latencies of 4—5 msec were common in response to stimulation of the spino-cervico-lemniscal path, whereas the dorsal-column responses of units in the zone of convergence generally showed a latency of 7 msec or more.

Discussion

The zone of convergence is a border zone between the somatotopically organized part of *n. centralis posterolateralis* (VPL) on one hand and *n. reticularis* (R), *n. lateralis posterior* (LP), *n. centralis lateralis* and *n. centralis anterior* on the other. It is located mainly within the morphological borderlines of VPL. This is clearly born out by the reconstructions based on the course of the identified penetrations and the location of the prussian blue spots in the histological sections. As shown in the diagram of Fig. 5 the zone of convergence may also include a narrow band of the neighbouring R and LP nuclei. The overlapping response may, of course, be explained as a spread of the electrical field set up in adjacent foci located in VPL, but it cannot be excluded that scattered islands of neurones in the border zone of R do in fact respond similarly to the neurones in the adjacent zone of VPL. Except for this border zone near VPL the reticulate nucleus did not respond to electrical stimulation of the spino-cervico-lemniscal path. This was clearly demonstrated by the lack of response from the penetrations located to R, but passing at some distance from the border with VPL.

Angel (1964) has recorded responses to high threshold cutaneous afferents from single thalamic units in the rat. These units were located in a region surrounding the ventro-basal complex, thus occupying a position similar to the zone of convergence. According to the author the region was identical with *n. reticularis thalami* and *n. centralis thalami pars dorsomedialis*. There are obvious differences between the thalamic units described by Angel and the neurones in the zone of convergence of the cat. Thus the former group did not respond to low threshold cutaneous stimuli, whereas the responses from the zone of convergence were evoked by volleys in low threshold, fast conducting paths. Other differences are the longer latency and duration of the response seen in the reticular neurones of the rat. It is therefore concluded that the two sets of observations are obtained from neurones engaged in different mechanisms.

Poggio and Mountcastle (1960) described thalamic neurones discharged by cutaneous afferents from hindlimbs and forelimbs. These neurones were located to the posterior group of nuclei (PO) which lacked somatotopical organization. The most anterior part of PO is according to Poggio and Mountcastle a thin curving lamella of tissue between VPL, corpus geniculatum laterale and the external medullary lamina. It projects forward to embrace the postero-dorso lateral aspect of VB' (the ventro-basal complex). The zone of convergence occupies a position in VPL that makes it tempting to look upon it as a rostral continuation of PO (cf. Fig. 5C). Several reasons do, however, indicate that the zone of convergence and the PO may belong to two different systems. One of these is the fact that the zone of convergence widens rostrally to the rostral pole of the lateral geniculate body (cf. Fig. 5B). Another is the lack of response to stimulation of the spino-cervico-lemniscal path in the more caudal parts of PO located to the border zone between the medial geniculate body and the ventrobasal complex (cf. Fig. 6). Convergence between the dorsal column path and the cutaneous path from the forelimb was observed in this region. The small responses, evoked by the spino-cervico-lemniscal path at this level, were, however, located more medially where the medial lemniscus enters the ventrobasal complex (cf. Fig. 5D and Fig. 6). Whitlock and Perl (1959) further showed that the posterior group of nuclei receive afferent input from elements of the anterolateral system of the spinal cord. The zone of convergence was, on the other hand, activated by low threshold spino-cervico-lemniscal and dorsal-column lemniscal paths. Responses evoked in the zone of convergence by stimulation of the

ventral quadrant of the spinal cord or to high threshold cutaneous afferents were not studied in the present series of experiments Such investigations are however, in progress

We have no direct knowledge concerning the type and location of the receptors activating the cells in the thalamic zone of convergence As shown by Gordon and Jukes (1963) and by Oswaldo-Cruz and Kidd (1964) the majority of the cells in *n cervicalis lateralis* are discharged from sensitive mechanoceptors with small cutaneous receptive fields on peripheral parts of the limbs Most of these cells in the lateral cervical nucleus send ascending axons into the medial lemniscus (cf Gordon and Jukes 1963) and sensitive well localized mechanoceptors on the limbs must therefore play a dominant role in the cervico thalamic input The same holds true for the dorsal-column lemniscal inflow It is, however, necessary to consider also a contribution from receptors in deep structures Andersson (1962) has shown that such afferents reach the cerebral cortex (SII) via both of the above mentioned paths, and Norrsell and Wolpow (1965) find that stimulation of high threshold muscle afferents evokes a response in SI and SII via both paths

The shorter latency of the thalamic response to electrical stimulation of the spino-cervico-lemniscal paths is in agreement with Mark and Steiner (1958) and with Norrsell and Voorhoeve (1962) The response to stimulation of the dorsal column showed a longer latency when recorded in the zone of convergence than was observed in the somatotopically organized part of VPL The difference was about one millisecond, which would allow time for another synapse in the dorsal column path to the zone of convergence It is therefore possible that the cells in the zone of convergence receive dorsal column excitation via the cells of the somatotopically organized region The possibility of convergence already at the level of the *n cervicalis lateralis* is excluded by the disappearance of the thalamic response to dorsal column stimulation after the hemisection just rostral to CI (cf Fig 2)

The dominating evidence of convergence must not conceal the fact that a considerable number of the cells in the zone of convergence responded only to stimulation of the spino-cervico thalamic path (cf Table 1) As mentioned above recording sites were observed, where a focal potential was evoked only by this path thus indicating the existence of nuclei of cells dominated by the spino-cervico-lemniscal inflow It is possible that these cells represent a system differing in function from that of the cells with convergence The features of this function are unknown but further studies concerning the cortical projections of the two populations may contribute valuable evidence

The relations between the zone of convergence and the adjacent thalamic reticular nucleus is another problem of great importance for the understanding of its function The existence of populations of neurones with great convergence located in the border zone between the relay nuclei and the reticular formation was previously observed by Gordon, Landgren and Seed (1961) and by Eisenmann Landgren and Novin (1963) in different parts of the trigeminal nucleus It is possible that such regions provide important connections between the specific projection paths and the unspecific reticular activating systems According to Rose (1950) and Hassler (1955) the thalamic reticular nucleus projects to restricted parts of the cerebral cortex The location of the cortical projection field is similar to that of the underlying thalamic nucleus The possibility therefore exists that the zone of convergence may be involved in reticulo-cortical mechanisms with local projection to the sensorimotor cortex

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From the Laboratory for the Theory of Gymnastics, University of Copenhagen, Denmark

The Form and Function of the Loaded Human Spine

By

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Abstract

Klausen, K. *The form and function of the loaded human spine* Acta physiol scand 1965 65 176—190 — The reaction of the spine and its muscles to an increase of the pull of gravity was investigated in different ways. The change of the curves of the spine was measured with an inclinometer The placement of the line of gravity in relation to L4 and the ankle joints was determined, and the activity of the trunk muscles and some of the leg muscles was investigated electromyographically From a discussion of the mechanical effect of gravity on the individual joints, and on the spine as a whole it is concluded that the short, deep muscles of the back must play an important part in stabilizing the individual joints, and that the long back muscles or the abdominal muscles are responsible for the stabilization of the spine as a whole. It was found that an increased pull of gravity always was counteracted by increased activity in one set of muscles only, i e either in the back muscles or in the abdominal wall muscles An increased pull of gravity gave rise to a flattening of the lumbar lordosis probably due to a deformation [illegible] counteracted by the psoas muscle

The weight-carrying function of the human spine and its muscles has been the subject of many experiments and discussions The muscles acting on the spine have been the subject of numerous investigations especially in the last 25 years (for references see Joseph 1960 and Basmajian 1962), and also the stabilizing effect of the ligaments has been investigated by different methods (Lucas and Bresler 1960, and Basmajian 1961) Asmussen (1960) found that the pull of gravity on the spine during normal standing always is counteracted by one set of muscles only, i e either by the back muscles or by the abdominal muscles, a view which is in contrast to most former concepts

The aim of the present studies is to find out how the erect human spine counteracts an increase of the vertical load For this purpose anthropometric measurements and gravity line determinations are compared with electromyographical records of the activity of the trunk muscles while standing unloaded, and with a load added to the weight of the body in different ways [1]

[1] *Part of this investigation has been shortly mentioned in 1962 (Asmussen and Klausen)*

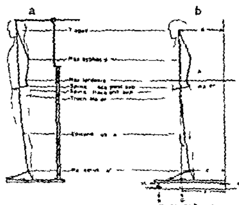


Fig 1 a Schematic drawing showing the "indicator" and the different measuring points
b Schematic drawing showing a subject standing on the platform used for the determination of the location of the line of gravity (See text)

Subjects and methods

The subjects were 31 male students, age 19—28 years, mean weight 71 kg mean height 181 cm They wore only trunks and their own ordinary shoes during the experiments

The curves of the spine were measured with an inclitometer (Mølhave 1958 Asmussen and Heeboll Nielsen 1959) which allows the measuring of the inclination of any line connecting two points on the surface of the body The readings could be made with an accuracy of ± 1/2° The inclinometric measurements were performed on 24 of the subjects in the standing position unloaded and while carrying a load of 20 kg in each hand During the measurements the distance between the heels and the angle between the feet of the subjects were adjusted to respectively 5—10 cm and 25—40° and they were asked to look straight ahead (pos 1 and 2 in Fig 2)

The curves of the spine were defined by the inclination of the lines connecting the following points 1 The mid point of a horizontal line between the two spin. iliac, post sup 2 The deepest point of the lumbar lordosis 3 The highest point of the thoracic kyphosis and 4 Tragus. A line connecting spin iliac post sup and spin iliac ant sup was used for the determination of the changes of the pelvic inclination (Fig 1 a) These latter points were chosen in order not to disturb the subjects during standing Since a line connecting the two points is not situated in the sagittal plane the inclinations found should be corrected for this deviation Such a correction always increases the found changes but since it in no case exceeds 1/2° the directly measured inclinations are used

Further the inclination of the legs was measured using a line connecting the malleolus fibulans and epicondylus lateralis femoris for the inclination of crus and a line connecting epicondylus lateralis and trochanter major for the inclination of femur

An indicator apparatus (Fig 1 a) was used for tracing the mean position in the postural oscillations of tragus during standing (Mølhave 1958) By means of the "indicator it was found that all subjects after slight voluntary movements, as for instance standing walking involuntarily took up a standing position of high reproducibility even from one day to another The measurements were taken when a helper announced that the subjects during their postural movements passed this intermediate position The inclinations of the different segments showed high reproducibility from one day to another the deviation being at most 1 2° except in a very few cases, where the deviation approached 1° In most cases this initial position was maintained for only a short period It was found that already after about 30 seconds the intermediate position of the postural oscillations moved either forward or backwards. This observation is in agreement with the findings of Smith 1954 Mølhave (1958) and Sheldon 1963 The measurements were therefore performed during the first 30 seconds of a standing period From the distances between the chosen points and the corresponding inclinations a diagram of the subject is constructed (fig 1) which serves to follow the change in the arrangement of the body segments from unloaded to loaded standing position (i e pos 1 and pos 2

Determinations of the location of the line of gravity for the whole body in relation to the ankle joints were performed by the method of DuBois Reymond 1903 the pressure on the

platform being registered by a *strain gauge dynamometer* (Asmussen 1960) The principle of the determination is shown in fig 1 b which shows a longitudinal section of the platform and a subject standing on the platform An angle iron (ax) forms an axis on one end of the platform [illegible]

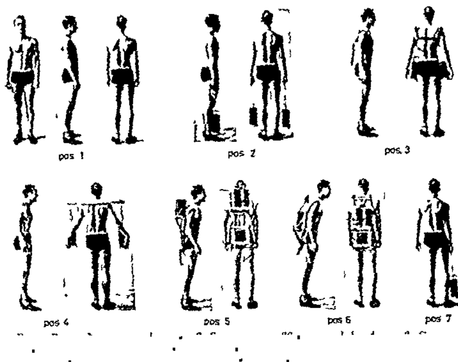
[illegible] because of their [illegible] of the action [illegible] units remotest [illegible] ained from the [illegible] because it is [illegible] the muscles

activity found is always alternating more or less due to the postural oscillations during standing Thus the estimation of the electrical activity found by means of surface electrodes will always be more or less subjective even if all the measurements are performed on the same [illegible] on different days it [illegible] is highly [illegible] om 5 000 [illegible] encies at [illegible] of course increase the uncertainty of the estimation of the muscular activity

Two types of electrodes were used The first consisted of small silver domes 10 mm in diameter as described by Floyd and Silver (1955) The other consisted of two small silver plates [illegible] distance between [illegible]

to the subject's left ankle [illegible] nected [illegible] ical [illegible] two [illegible] part of [illegible] lane as

Rectus abdominis At first the activity of this muscle was investigated at two [illegible] ls (just below the umbilicus and about 3 cm *below the processus xiphoideus*), but since the activity



at the two levels always was the same in the standing positions investigated the activity in most later cases was registered at only one level (just above umbilicus)

Obliquus abdominis externus The electrodes were placed so that one of the silver plates was about 4 cm above the highest point of crista iliaca and the other ventrally to and about 2 cm below the first one

Iliopsoas An attempt was made to register the activity by means of the silver domes described

eliminate possible errors from m sartorius by registering its activity simultaneously with the registration from iliopsoas The electrodes were placed ventrally over the most prominent part of the upper third of m sartorius.

The activity of the following leg muscles was also investigated rectus femoris vastus medialis biceps femoris, medial flexors glutaeus maximus glutaeus medius, tibialis anterior soleus gastrocnemius and peroneus longus The activity in these muscles will not be discussed in detail

responding positions in Fig 2

All the determinations were performed on a subject on a given day and each subject was investigated on at least two different occasions

TABLE I

Subject	Erector spinae lumbar portion		Erector spinae thoracic portion		Rectus abd		Obliq ext	
	pos 1	pos 2	pos 1	pos 2	pos 1	pos 2	pos 1	pos 2
HJLH	+	++	+	++			+	+
EJ	+	++		+				+
AR	+	++		+				+
BP	+	++	+	++				+
FH	+	++	+	++				+
JW	+	++		+			+	+
MD	+	++	+	++			+	+
HGJ	+	++	+	++			+	+
PCE	+	+−	+	+			+	++
OF	+	++	+	++			+	+
KG	+	++	+	++				
AMN	+	++	+	++				+
JQ	+	++	+	++				
OT	+	++	+	+				+
PQ	+	++	+	++			+	+
PLT	+	+	+	++				+
IA	+	++	+	+				+
OSK	+		+	++			++	++
NWP				+	+	++	+	++
JD	+			+		+	+	++
JFA				+			+	++
OEA			+	++			+	++
PM			+	++	+	++	+	++
JH	+		+	++		+	+	++

+ Indicates activity in the muscles ++ indicates that the activity found in pos 2 is greater than that found in pos 1 +− indicates that the activity found in pos 2 is less than that found in pos 1 and no sign indicates that there is no measurable activity in the muscles investigated

Results

a EMG findings

In Table I an attempt is made to give an account of the activity found in the trunk muscles while the subjects were standing in pos 1 and pos 2 As can be seen the material can be divided into two main groups according to the activity in the lower back muscles when standing with 20 kg in each hand (pos 2) Seventeen of the 24 subjects showed marked activity in the lower back muscles in this position These will henceforth be referred to as group I The subjects PCE and PLT did not show an increase of the activity in the lower back muscles from pos 1 to pos 2, but they still showed a marked activity in pos 2

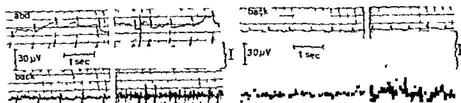


Fig 3 Typical electromyograms of a subject from group I and from group II Left parts Standing without load, right parts With 20 kg in each hand The electrocardiograms are visible in the silent muscle groups

On the average the mean electrical activity in pos 2 was doubled as compared with the activity when standing in pos 1 In this position the integrated mean activity of the lower back muscles, measured in 11 of the 17 subjects in group I, was found to be 7—8 μV, and in pos 2 13—14 μV in each side This is a very small degree of activity For instance, it was found on the same subjects that the integrated activity in the lower back muscles when bending slightly forward was about 50—60 μV in each side, and

this amount of electrical activity again represents only a small fraction — approximately one tenth — of the maximum obtainable from the muscles Since a rectilinear relation ship exists between the integrated electrical activity and the mechanical tension developed in human muscles (Lippold 1952, Edwards and Lippold 1956, and others), it must be assumed that the tension produced by the back muscles both in pos 1 and in pos 2 represents only a very small fraction of their maximal strength

Seven of the 24 subjects did not show any measurable activity in the lower back muscles in pos 2 This group (henceforth called group II) is not as homogeneous as group I 3 out of the 7 showed a slight activity in the lower back muscles in pos 1 but none in pos 2, and in two subjects there was no activity, neither in rectus abdominus, nor in the lower back muscles, but only in obliquus externus both in pos 1 and pos 2 Here it is worthy of note that both the rectus abdominus and the obliquus externus muscles are able to prevent the trunk from falling backwards As can be seen from table I activity in the oblq ext also occurs in some of the subjects in group I This activity however is very small compared to the activity found in the subjects of group II the mean voltage being only 1/5 to 1/7 of that in group II in both pos 1 and pos 2 It seems therefore, reasonable to characterize the 7 subjects in group II by their activity in the abdominal wall muscles i e in both rectus abdominus and obliquus externus In similarity to what was the case for the back muscles in group I, the activity of the abdominal wall muscles in pos 1 represents only a very small fraction of the maximally obtainable activity In pos 2 it is increased to about double the value of that in pos 1, in a few cases tripled Examples of the distribution of muscular activity in the two groups I and II with and without load (20 kg) are given in Fig 3

The classification in group I and II and its percentage distribution agrees on the whole with Asmussen s findings (1960), and is valid for most of the subjects not only in pos 1 and pos 2 but also in pos 7 i e when standing with 20 kg in only one hand In this position the subjects in group I counteract the increased pull of gravity e g to the left by an increased activity of the back muscles and the oblq ext in the right side while in the subjects in group II the activity is increased mainly in the rectus

TABLE II

		Interspinal max lordosis	Max lordosis-max kyphosis
Pos 1	Group I	degrees	degrees
	n = 17	+ 10 0	− 9 8
	se ±	1 2	1 0
	Group II		
	n = 7	+ 7 2	− 11 2
	se ±	1 5	1 5
Pos 2	Group I		
	n = 17	+ 8 1	− 6 7
	se ±	1 1	1 1
	Group II		
	n = 7	+ 5 7	− 8 3
	se ±	1 3	1 8
Change of inclinations from pos 1 to pos 2	Group I	− 1 9	− 3 1
	n = 17	0 4	0 4
	se ±		
	Group II		
	n = 7	− 1 4	− 2 9
	se ±	0 6	0 7

The values are given in degrees + indicates inclinations forward and — inclinations back dicates an increase and — a decrease

abd and oblq ext There is no fundamental difference in the activity of the thoracic portion of the back muscles between the two groups All subjects show a marked activity when standing in pos 2 and most of them more or less activity also in pos 1 The mean voltage for 12 subjects was found to be about 6—7 μV in pos 1 and about 25 μV in pos 2 in each side This should indicate a quadruplication of the muscular activity from pos 1 to pos 2

b The curves of the spine

Mean values for the inclinations of the different segments of the vertebral column and the lower extremities are presented in Table II The material is divided into two groups according to the electromyographical findings

As can be seen from the standard errors the scattering of the individual values around the mean is considerable The small differences between the two groups can, therefore, only be taken to indicate a trend (Only the change of the pelvic inclination from pos 1 to pos 2 is significantly different for the two groups) Diagrams of the mean values for the two groups of subjects are presented in Fig 4 From this it appears that there is no difference between the two groups in the way the mutual relations of the dif

Max kyphosis tragus	Trochanter *major tragus*	Crus	*Femur*	' Inclinatio *pelvis"*
degrees	degrees	degrees	degrees	degrees
+ 21 4	+ 2 0	+ 7 0	+ 3 7	+ 8 8
0 9	*0 4*	*0 6*	0 5	*0 8*
+ 19 4	+ 1 3	+ 6 9	+ 4 0	+ 8 1
1 2	0 7	0 8	1 0	1 0
+ 24 0	+ 3 4	+ 5 7	+ 3 1	+ 6 7
0 9	0 4	0 6	0 7	0 8
+ 21 9	+ 2 6	+ 5 6	+ 3 4	+ 9 3
1 2	1 2	1 2	1 2	1 3
+ 2 5	+ 1 4	− 1 4	− 0 6	− 2 1
0 4	0 2	0 2	0 5	0 2
+ 2 5	+ 1 0	− 1 4	− 0 6	+ 1 2
0 8	*0 4*	*0 7*	0 3	0 5

wards relative to the vertical For the changes of the inclinations from pos 1 to pos 2 − in

ferent segments change from pos 1 to pos 2 This is further illustrated in the diagrams in Fig 5 Here the inclinations of the different segments of the vertebral column are drawn with the measuring point at the maximal lordosis as fixed point In both groups there is a slight relative movement forward of the spine from the *unloaded* (*pos* 1) to the loaded position (pos 2) so that the tragus moves forward relatively to the fixed lordosis But from Fig 4 it can be seen that the increased inclination forward of the spine is compensated by a corresponding decrease in the inclination of the lower extremities so that the position of tragus in space remains practically unaltered

In Table III an attempt is made to express the curves of the spine in degrees The sum of the inclinations of the lines spin iliac post sup-deepest point of lumbar lordosis and deepest point of lumbar lordosis highest point of thoracic kyphosis is called lordosis , and the sum of the inclinations of the lines deepest point of lumbar lordosis highest point of thoracic kyphosis' , and highest point of thoracic kyphosis-tragus is called kyphosis (cf Asmussen and Heeboll Nielsen 1959 As can be seen there is no significant difference between the two groups in the magnitude of the curves, and also the change of the curves from pos 1 to pos 2 is the same In both groups loading produces a flattening of the lordosis' while the kyphosis is unchanged



Fig 4

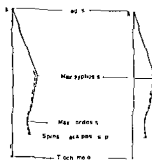


Fig 5

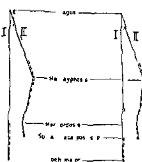


Fig 6

Fig 4 Mean values of the inclinations of the different segments of the body Full lines Standing without load (pos 1) Dashed lines Standing with 20 kg in each hand (pos 2)

Fig 5 Diagram showing the inclinations of the different segments of the spine in the two groups Full lines Standing without load (pos 1) Dashed lines Standing with 20 kg in each hand (pos 2) Note In both groups a slight forward shift of the spine from pos 1 to pos 2

Fig 6 The inclinations of the different segments of the spine in pos 1 and 2 Full lines mean values for group I Dashed lines mean values for group II Note that the forward inclination in group II is less than that in group I, in both pos 1 (unloaded) and pos 2 (loaded)

TABLE III

Subjects	Pos 1		Pos 2		Changes of curves from pos 1 to pos 2	
	Mean	± Se	Mean	± Se	Mean	± Se
	degrees		degrees		degrees	
"Kyphosis						
Group I	31 3	1 2	30 8	1 5	− 0 6	0 5
Group II	30 6	1 5	30 2	2 5	− 0 4	1 3
Lordosis						
Group I	19 9	1 3	14 9	1 4	− 5 0	0 6
Group II	18 4	2 6	14 0	2 8	− 4 4	0 5

Even though there is no difference in the spinal curves of the two groups there is how

forward inclination of the spine is smaller than in group I both when stan s loaded and when standing with 20 kg in each hand

TABLE IV

	Erector spinae lumbar portion				Erector spinae thoracic portion				Rectus abdominis				Obliquus externus				Iliopsoas			
Pos	1	4	5	6	1	4	5	6	1	4	5	6	1	4	5	6	1	4	5	6
Subjects																				
KK	+	++	++	+−	+	+	+−	+−							+					+
KJ	+	++	++	++	+		+−	+												
RM	+	++	+	+	+	++		+							+					+
HN	+	++	++	+−	+	+	+	+									+			+
ED	+	++	++	+−	+	+	+	+							+			+	+	+
OL	+	++	++	+−		+								+	+	+				+
GM	+	++	++	+−	+	+	+	+						+	+	+	+	+	+	+

The location of the line of gravity for the whole body in relation to the ankle joints was determined in all 24 subjects For the subjects in group I the mean values were 6 0 cm (se = ± 0 3) in front of the ankle joints in pos 1 and 6 3 cm (se = ± 0 4) in pos 2 The mean values for group II were 5 7 cm (se = ± 0 7) in pos 1 and 5 6 cm (se = ± 0 6) in pos 2 These values are in close agreement with Hellebrandt s ob

group I and slightly backwards in group II This impression is further strengthened by the conditions found in the soleus and gastrocnemius muscles where on the whole the subjects in group II showed less activity in both pos 1 and pos 2 than did group I

Determinations of the location of the line of gravity for the head arms and trunk in relation to the *center of the body of the lumbar vertebra (L4)* were performed in 12 of the 17 subjects in group I The mean value in pos 1 was 1 5 cm (se = ± 0 4 *dorsally* to the center of L4 and in pos 2 1 2 cm (se = ± 0 5) *ventrally* to the center of L4 In 3 of the 7 subjects in group II the mean value was 0 6 cm *dorsally* to the center of L4 in pos 1 and 1 3 cm *dorsally* to the center of L4 in pos 2 Although these measures are rather scanty there seems to be a trend for the line of gravity for the head trunk and arms to move forward on loading in group I and slightly backwards in group II

The activity of the trunk muscles while standing in pos. 3—6 (see Fig 2) was investigated in 7 subjects (not included in the original 24) *The activity in pos 3* was investigated in order to see if a vertical elevation of the center of gravity of the load (40 kg) would affect the weight-carrying function of the spine and its muscles No difference between the muscular activity in pos 2 and pos 3 was found and the results are therefore omitted in Table IV The activities found *in pos 4—6 are presented* in Table IV using the same signatures as in Table I

The EMG s were not integrated and therefore no mean voltages can be given However, the degree of activity can be estimated first of all from the size but also from the frequency of the recorded action potentials The activity of the lumbar back muscles in pos 4 was considerable in all subjects On an estimate it was about four times the activity found in pos 1 (Three of the subjects of the original group II, JD OEA

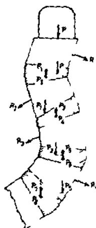


Fig 7

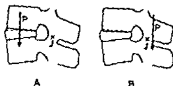


Fig 8

Fig 7 Part of segmental curved column composed of alternating rigid and elastic (hatched) elements Axes of rotation are the points in the middle of the elastic elements The rotatory effect of a vertical load (P) on the different segments is indicated by the arrows (R_1—R_4)

Fig 8 The effect of gravity on an intervertebrate disc in the lumbar spine A the line of gravity passes ventrally to the inter articular joint (J) B the line of gravity passes dorsally to the joint

and PM were also investigated in pos 3 and 4 There was no difference in the distribution of muscular activity between pos 2 and 3, but in pos 4 all three subjects showed marked activity in the back muscles while the abdominal muscles became silent) In pos 5 the activity of the back muscles was as large as in pos 4 except in one subject In pos 6, however, the activity was quite different In almost all cases the activity of the lumbar back muscles was decreased, even as compared to the activity in pos 1 and it was the only position, where a considerable activity in the iliopsoas could be registered in all but one of the subjects

The activity of the back muscles over the thoracic kyphosis was more variable On the whole the activity in pos 4 and pos 1 was the same while in pos 5 and 6 there was a slight decrease

Discussion

The effect of gravity on the different joints of the body is usually predicted from the placement of the 'line of gravity' in relation to the axis of the joints the line of gravity being the vertical line through the center of gravity for the part of the body above the joint under discussion As will appear from the following example this is only permissible when no movements take place in the joints above the joint investigated

If a vertical load is applied to a segmental curved column e g a segment of the spine composed of alternating rigid and elastic elements the effect in the different movable joints will be as shown in Fig 7 (leaving the weight of the segments out of account) As can be seen the effect of the force P on the joints will be a rotation the direction of which is only dependent on the mutual relations of the axes of rotation in the joints and not on the point of application of the force P That means that except for the first joint, the effect on the joints will be independent of the antero posterior position of the load on the top of the column According to this gravity — i e the weight of the head arms and trunk—should always tend to increase the curves of the spine

However, in a given situation this tendency is counteracted by a complex system of stabilizing forces due to the compression of the intervertebrate discs tension in the

different ligaments activity of the trunk muscles and probably also the intrathoracic and intra abdominal pressures (Morris *et al* 1961) A rotatory effect of the force P on the uppermost joint will for instance be counteracted, partly by muscular forces acting on the other side of the axis of rotation and partly by the compression of the intervertebrate disc If the top joint is stabilized in this way, the force P, through its point of application, will have a more direct effect on the next joint which effect in turn may be counteracted by other muscles etc If we imagine the stabilization of the joints continued in this manner down through the spine, it can be seen, that the effect of a vertical load on any particular joint will depend on the position of the line of gravity for this load in relation to the axis of rotation of the joint, i e the effect on each particular joint of the spine will be dependent on the placement of the line of gravity for the part of the body which lies above the joint

The stabilizing effect of the muscles is most probably due to contraction of the deep one-joint muscles of the back, and the activity in these muscles will increase, corresponding to the increase of the pull of gravity As mentioned by Asmussen and Klausen (1962) an increase of the two forces — the pull of gravity and the contraction of the deep back muscles — will cause an increase of the compressive forces on the intervertebrate discs, and since these discs in the lumbar portion of the spine, are highest ventrally the absolute deformation will also be largest ventrally, and thus cause a decrease of the lumbar lordosis The erect position of the stabilized spine as a whole in relation to e g the lumbo-sacral joint is maintained by contraction of the more superficial long back muscles if the line of gravity passes ventrally to the axis of movement in this joint and an increase of the pull of gravity will cause an increase of the activity in these muscles If the line of gravity passes behind the axis of movement, muscles lying in front of the spine will be activated viz the muscles of the abdominal wall

From the above it is easy to see, why the increase of muscular activity from pos 1 to pos 2 in the thoracic portion of the back muscles is much greater than in the lumbar portion since the line of gravity is placed further ventrally in relation to the joints in the thoracic part of the spine than in the lumbar spine The unaltered curvature of the thoracic kyphosis may partly be due to the fact that the thickness of the intervertebrate discs in this part of the spine is almost the same ventrally and dorsally, and partly to the stabilizing effect of the thorax

From this point of view it becomes obvious that it is important to establish the precise location of the frontal axis of movement between two vertebrae According to Dittmer (1930) the axis in the lumbar part of the spine is situated dorsally in the intervertebrate discs Wiles (1935) however found that in the erect position the axis passes through the centers of the joints between the articular processes The last position seems most probable from a teleological point of view since it will cause a minimum of stress on the rather weak interarticular joints If the frontal axis passes through these joints it can be seen from Fig 8 that with the line of gravity placed ventrally to the joint the torque on the joint can be counteracted both by contraction of e g the interspinal muscles and by compression of the intervertebrate discs According to Lucas and Bresler (1961 the critical load for the isolated erect ligamentous spine in the frontal plane is about 33 kg which is just below the weight of an average torso Assuming the same critical load in the sagittal plane this may indicate that most of the torque on the interarticular joints in pos 1 is counteracted by compression of the discs while in pos 2 the increased pull of gravity must demand an increased stabilizing effect of the deep one joint muscles

It was found that about 25 % of the subjects did not show any measurable activity in the back muscles, when standing with 20 kg in each hand (pos 2), and that apparently in these cases the erect position of the spine was maintained by activity in the abdominal wall muscles This should indicate that the line of gravity for these subjects is placed dorsally to the lumbar part of the spine From Fig 8 it is evident that if the line of gravity is placed dorsally to the interarticular joints the torque cannot be counter acted by compression of the intervertebrate disc The stabilization of the joint in this situation may be due partly to the stretching of anulus fibrosus and partly to muscular activity It is, however difficult to see, which muscles are able to stabilize the joints under these circumstances, since activity in the deep one joint muscles of the back would increase the torque on the joints Owing to the anatomical arrangement of the abdominal wall muscles these must be presumed to be able only to prevent the whole stabilized spine from falling backwards and not to stabilize the individual joints It is possible that the psoas muscles by a differentiation of the activity in their different parts are able to stabilize the individual joints but in almost all cases no activity was found with certainty in these muscles in pos 2 Another possible explanation is that the stabilization is established by contraction of some of the deep transverso-spinal muscles. According to Molbech (1964) and others the effect of two-joint muscles may be paradoxical The effect of the gastrocnemius on the knee will for instance within a certain range of knee flexion be an extension provided the position of the ankle joint is fixed and the movement of the hip-joint is limited to a more or less vertical plane By means of X ray pictures of the lumbar spine in the standing position it can be shown that if the different conditions formulated by Molbech are fulfilled the deep two- (and three) joint muscles of the back will be able to counteract the backward pull on the interarticular joints (cf Fig 8) The EMG s did not show any activity in the back muscles when activity occurred in the abdominal wall muscles but this is maybe because the surface electrodes were incapable of picking up action potentials from the deepest part of the back muscles Further investigations e g by means of needle electrodes will be necessary to confirm this hypothesis Also further investigations on the location of the line of gravity are necessary since the present measurements are incomplete In this connection it must be pointed out that in the determination of the line of gravity in relation to L4 the weight of most of the viscera in the abdominal cavity was included in the weight of the body above L4 This may cause an error in the determination of the location of the line of gravity since part of the weight of the viscera must be supported by the pelvis and thus probably has no effect on the spine If so the line of gravity may be located more dorsally than found in the present experiments

The difference in the change of the pelvic inclination between the two groups of subjects can be explained neither from the activity in the hip joint muscles nor from the location of the line of gravity in relation to the hip-joints From a teleological point of view however the changes in inclination must be considered to be advantageous since a decrease of the pelvic inclination will cause an extension of the superficial erector spinae muscles and an increase of the inclination will cause an extension of the abdominal wall muscles According to this the ability for the two groups of muscles to develop tension should be increased by the change of the pelvic inclination from pos 1 to pos 2

In pos 4 the load of 40 kg is held by means of a yoke over the shoulders As can be seen from Fig 2 this causes a slight stooping and forward inclination of the spine which is compensated by a decrease of the inclination of the lower extremities This

change in the mutual orientation of the body segments gives rise to a forward shift of the line of gravity, which again is compensated by an increased activity of the back muscles Practically no activity occurs in the abdominal wall muscles

In pos 5 and 6 the load is attached to a carrier, high and low on the back, respectively

The orientation of the trunk — i e the spine — in pos 5 is almost the same as in pos 4, except for the stooping Since the activity in the lumbar portion of the back muscles is almost the same in the two positions, the placement of the line of gravity in relation to the lumbar lordosis must be assumed to be almost the same in pos 5 as in pos 4 On the other hand, the attachment of the carrier to the back, by means of straps around the shoulders, will cause a slight pull backwards in the thoracic part of the spine, and thus give rise to a decrease in the activity of the back muscles at that level As can be seen from Fig 2, the forward inclination of the trunk is not counteracted by a decrease of the inclination of the lower extremities in pos 5 On the contrary, the forward inclination of the legs is increased, so that the location of the vertical projection of the center of gravity for body and load remains undisturbed in relation to the ankle joints (Hellebrandt *et al* 1944, Thomas 1959)

In pos 6 the load is placed low on the back In this position the activity in the back muscles is decreased both in the lordosis and over the kyphosis, in spite of the fact that the forward inclination of the trunk is greatly increased, in order to keep the combined center of gravity of the body plus the weight in the same position The decreased activity of the back muscles in this position might be explained as the result of a backward shift of the combined center of gravity for the load plus trunk in relation to L4 On the other hand it seems that the forward inclination of the trunk in pos 6 is maintained actively by contraction of the psoas muscle According to this the decreased activity of the back muscles may be explained by the increased pull backwards of the load via the straps around the shoulders

I want to express my gratitude to the Testing and Observation Institute of the Danish National Association for Infantile Paralysis where part of this investigation was performed

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Inhibition of Gastric Secretion by Acid in Proximal and Distal Duodenal Pouches

By

Sven Andersson, Göran Nilsson and Borje Uvnäs

In 1961 Andersson and Uvnas showed that acidification of an isolated duodenal bulb produced a pronounced inhibition of postprandial secretory responses from Pavlov pouch dogs The present experiments were undertaken (1) to determine the effect of duodenal bulb acidification on gastric acid secretion induced by other stimuli such as gastrin and histamine and (2) to determine the intraduodenal extension of the pH sensitive inhibitory mechanism

Methods

11 mongrel dogs weighing 12—17 kg were prepared as follows At an initial operation 5 dogs were provided with Pavlov pouches and 6 dogs with Heidenhain pouches 3 to 4 weeks later a

basal gastric output was recorded for 1 hr and secretory responses collected in 15 min periods The volume was measured and the acidity was titrated with 0 01 N NaOH and phenolphtalein as indicator Gastrin was prepared from hog

observed for 1 to 1 1/2 hrs. After the period of duodenal acidification the secretory responses were followed during another period of 1—2 hrs

Results

The effect of acid in the pouch of the duodenal bulb on the response to gastrin was studied in 5 Pavlov and 4 Heidenhain pouch dogs In all experiments a pronounced inhibition of acid output was observed the degree of inhibition varying between 60 and 100 per cent (Fig 1 A) Acid was instilled into the distal pouch of the duodenum in 3 Heidenhain pouch dogs No inhibition of gastrin stimulated secretion was observed (Fig 1 B) The effect of acid in the duodenal bulb on the secretory responses to histamine was studied in 2 Pavlov and 2 Heidenhain pouch dogs. No significant inhibition could be detected

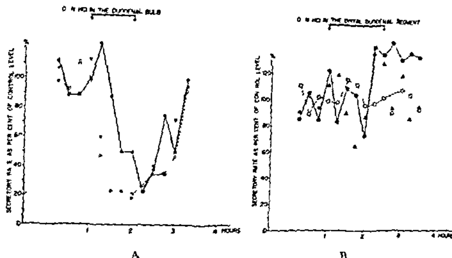


Fig 1 Effect of HCl in duodenal bulb (A) and distal duodenal segment (B) on gastric secretory response to gastrin in Heidenhain pouch dog Each curve represents one experiment The 100 per cent level is the mean 15-min gastric secretion during the hour before the infusion of acid

Discussion

The present results compare favorably with the previous observation that duodenal bulb acidification exerts a marked inhibition of gastric acid secretion (Andersson and Uvnäs 1961) The present experiments show that the bulbar mechanism is highly specific in inhibiting the secretory response to gastrin but not to histamine Our finding that acid in the distal segment of the duodenum was ineffective in inhibiting the response to gastrin is further evidence for the hypothesis that the inhibitory mechanism is located mainly in the bulbar mucosa It is however possible that partial secretory inhibition can be achieved from a transitional area located in the duodenal segment interposed between the entrances of the bile and pancreatic ducts which has not been studied in this investigation

Recently Andersson and Grossman (1965) have found that the content of duodenal bulb in man often has a much higher acidity than that observed in the postbulbar duodenum Values below pH 2 were not uncommon In preliminary studies we have found that a bulbar pH of 2 to 2 5 is sufficient to inhibit the secretion Combined these observations strongly suggest that inhibitory influences arising from the duodenal bulb could play an important role in the normal regulation of gastric secretion

This work was supported by grants from Statens Medicinska Forskningsråd U 306 W 303 Karolinska Institutets lärarkollegium Svenska Sällskapet för medicinsk forskning and Torsten och Ragnar Söderbergs Stiftelse

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On the Variation of $D_{L_{CO}}$ with Increasing Oxygen Uptake during Exercise in Healthy Ordinarily Untrained Young Men and Women

By

U. FREYSCHUSS and A. HOLMGREN

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Abstract

Freyschuss U and A Holmgren. *On the variation of* $D_{L_{CO}}$ *with increasing oxygen uptake in healthy, ordinarily untrained young men and women* Acta physiol scand 1965 65 193—206 — $D_{L_{CO}}$ was measured at rest in supine position and during steady state exercise in sitting position at increasing oxygen uptake in 9 women and 9 men using the steady state technique. $D_{L_{CO}}$ was found to increase on transition from supine rest to sitting work increase in 4 of 5 men and 1 of 8 women In 7 women there were only minor changes On increasing the work load, $D_{L_{CO}}$ was found to reach an approximately constant level at a heart rate of 120 beats per minute This value for $D_{L_{CO}}$ may be called a true "maximal" $D_{L_{CO}}$ and was found to be significantly correlated to various measures of body size, the size of the cardiovascular system and the size of the lungs

The diffusing capacity of the lungs for carbon monoxide ($D_{L_{CO}}$) expresses the conductance of the lungs for CO, i e the flux of carbon monoxide, $\dot{V}_{CO}$ ml STPD min^{-1} per unit driving pressure, ($P_{A_{CO}}$—$P_{\bar{c}_{CO}}$) mm Hg (Krogh 1915, Forster 1957) $D_{L_{CO}}$ is dependent on such factors as the size of the diffusion surface of the lungs, the permeability and thickness of the diffusion membrane It has been found to vary with such variables as body size, sex, age, degree of physical fitness of the subject, degree of inflation of the lungs and alveolar oxygen tension (cf Forster 1957)

The increase of $D_{L_{CO}}$ during exercise was first demonstrated by Krogh (1915) and verified by Boje (1933) Since then a large number of studies have been performed utilizing both the single breath method of Krogh, as modified by Ogilvie *et al* (1957),

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases

and the steady state technique introduced by Filley, MacIntosh and Wright (1954), and Bates, Boucot and Dormer (1955)

Filley and coworkers (1954) found in healthy young subjects that during lower grades of exercise in upright position, $D_{L_{CO}}$ increased to about twice the resting value but showed no significant tendency to increase further during more severe grades They concluded that when $D_{L_{CO}}$ was measured during exercise of a severity of about 4 times resting oxygen uptake, a measure of maximal $D_{L_{CO}}$ was obtained The existence of a maximal niveau for $D_{L_{CO}}$ ($D_{L_{CO\,max}}$) has since then been studied and disputed using both CO and oxygen as test gases, Riley *et al* (1954), Cugell *et al* (1956), Shepard *et al* (1958) Turino *et al* (1963)

The obtained results have varied with respect to whether D_L levels off to a maximal niveau during exercise or not This is true both for studies using oxygen and carbon monoxide as test gases Riley *et al* (1954), found that $D_{L_{O_2}}$ reached a level at $V_{O_2} > 1000$ ml min⁻¹, while Turino *et al* (1963) found that $D_{L_{O_2}}$ increased almost linearly with V_{O_2}

Regarding $D_{L_{CO}}$, Linderholm (1959), Shepard *et al* (1958), Donevan *et al* (1959), and Mostyn *et al* (1963) found a progressive increase with increasing V_{O_2} According to Donevan *et al* (1959), $D_{L_{CO}}$ can be regarded as a linear function of V_{O_2} Studies using single breath technique have also reported similar results (Ogilvie *et al* (1957)

Turino *et al* (1963) reported that $D_{L_{CO}}$ increased successively less with increasing oxygen uptake

The great majority of published reports thus seem to show that D_L increases continuously without reaching a niveau, with increasing V_{O_2} using both oxygen and CO [illegible] the results reported [illegible] (1963) demonstrate

The present investigation was undertaken to try to establish with the aid of multiple measurements during exercise with increasing load, whether a maximal niveau of $D_{L_{CO}}$ is usually reached and if so at what relative work load this occurs The results were also used to relate such a measure of 'maximal' $D_{L_{CO}}$ to various anthropometric, circulatory and pulmonary parameters to collect further information of the factors that influence 'maximal $D_{L_{CO}}$', determined by the steady state method in healthy untrained young subjects

Methods

The methods used in the present investigation have been reported earlier in two reports on errors envolved in determination of $D_{L_{CO}}$ (Holmgren 1964 a b) and shall only be mentioned [illegible]

helium dilution method using a closed spirometer system *Dynamic lung volumes* 1, BTPS were determined with a modified Bernstein spirometer

Fractions of gases, F_{O_2} and F_{CO_2}, were analyzed with Scholander technique *Gas volumes* were measured with a spirometer

The methodological errors in the present investigation have been analyzed separately and the reader is referred to Holmgren (1964) for the details All computations were performed with an IBM 1401 digital computer

Statistical methods Statistical treatment of the result were made using standard procedures The following conventions for significance levels were used $P < 0\,001$, highly significant, $P < 0\,01$, significant, and $P < 0\,05$, almost significant The multiple regression analyses were performed with the aid of an IBM 7090 computer as were most of the linear regression analyses

Material and procedure

sometimes with a low load

The subjects arrived at the laboratory early in the morning after a standardized light meal. A catheter was introduced into the left brachial artery with a percutaneous technique and,

Results

Total hemoglobin, THb, averaged 471 g (SD = ± 55 g) for the women, and 659 g (SD ± 102 g) for the men, corresponding to 8 2 and 10 1 g/kg body weight, values of the order as presented earlier with the same technique (Holmgren *et al* 1957) The regression

TA[illegible] [illegible] > 120 ml STPD [illegible] mmHg⁻¹ related to height [illegible] weight) [illegible], BSA [illegible] THb, g blood volume, l heart volume [illegible] C, l BTPS TC, l BTPS FRC, l BTPS, Midcapacity l BTPS [illegible] FEV 1.0 l BTPS in 18 healthy young ordinarily trained men and women [illegible] value and S.D. [illegible] = 7.8 ml [illegible], mmHg⁻¹ or = 21.6 per cent of the [illegible]

	Independent variable	Regression equation	Residual S.D. Units	Residual S.D. % of mean	b/sb	r
Body dimensions	Height	y = 0.632 x − 73	5.60	14.4	4.09	[illegible]
	Weight	y = 0.410 x − 11	7.11	19.6	2.00	[illegible]
	(Weight) [illegible]	y = 1.53 x − 2.7	7.13	19.6	2.06	[illegible]
	BSA	y = 28.8 x − 14	6.30	17.4	3.16	[illegible]
Circulatory dimensions	THb	y = 0.0[illegible]4 x − 5.5	3.92	10.7	7.2	[illegible]
	BV	y = 7.56 x − 2.1	[illegible].78	16.0	3.9	[illegible]
	HV	y = 0.039 x + 11.4	6.60	18.2	2.8	[illegible]
	[illegible]	y = 0.020 x + 19.3	5.50	15.2	4.5	[illegible]
Pulmonary dimensions	VC	y = 7.52 x + 3.3	4.[illegible]4	12.5	[illegible].8	[illegible]
	TC	y = 5.53 x − 4.3	4.20	11.6	6.[illegible]	[illegible]
	FRC	y = 8.48 x + 12.2	4.6[illegible]	12.8	5.6	[illegible]
	MC	y = 8.12 x + 10.9	4.86	13.5	5.5	[illegible]
	(MC) [illegible]	y = 17.6 x − 1.4	4.87	13.5	5.2	[illegible]
	FEV 1.0	y = 6.6 x + 10.1	6.17	17.0	3.5	[illegible]

helium dilution method using a closed spirometer system *Dynamic lung volumes* 1, BTPS were determined with a modified Bernstein spirometer

Fractions of gases, F_{O_2} and F_{CO_2}, were analyzed with Scholander technique *Gas volumes* were measured with a spirometer

Carbon monoxide in gas phase was analyzed with a hopcalite CO-meter (Stålex) as described by

cf Holmgren 1964 a, b

The methodological errors in the present investigation have been analyzed separately and the reader is referred to Holmgren (1964) for the details All computations were performed with an

Material and procedure

The material consists of 9 healthy young women and 9 healthy young men living an ordinary sedentary life All had normal electrocardiograms and chest X rays

The general design of the experiment was such as to allow study of the $D_{L_{CO}}$ during steady

sometimes with a low load

The subjects arrived at the laboratory early in the morning after a standardized light meal. A catheter was introduced into the left brachial artery with a percutaneous technique and,

Results

Total hemoglobin, THb, averaged 471 g (SD = ± 55 g) for the women, and 659 g (SD ± 102 g) for the men, corresponding to 8 2 and 10 1 g/kg body weight; values of the order as presented earlier with the same technique (Holmgren *et al* 1957) The regression

TABEL I Some anthropometric data, of significance for dimensional evaluation of the oxygen

	Height, cm	Weight, kg	B S A m^2	THb, g	Blood vol l	Heart vol, ml	W_{170}, kpm/min
Women							
1	167 0	54 0	1 60	425	3 6	535	450
2	164 0	53 0	1 57	465	4 2	490	650
3	164 0	47 0	1 50	405	3 6	465	400
4	166 0	59 0	1 66	460	3 9	655	600
5	175 0	64 0	1 80	565	4 9	670	570
6	161 0	50 0	1 52	450	3 8	545	630
7	163 0	58 0	1 62	490	4 4	580	700
8	182 0	76 0	1 98	550	5 0	600	700
9	170 0	56 0	1 66	430	3 4	565	770
$\bar{x}$	168 0	57 4	1 66	471	4 09	567	608
S D	±6 7	±8 6	±0 47	±55	±0 57	±68	±120
Men							
1	175 0	66 5	1 83	590	5 0	760	900
2	189 0	64 5	1 93	685	5 3	695	950
3	191 0	73 5	2 05	860	6 1	710	1 200
4	169 0	63 0	1 74	485	4 2	570	950
5	177 5	71 5	1 90	670	5 1	835	1,300
6	173 0	50 5	1 62	645	4 9	545	700
7	183 5	72 5	1 96	730	5 0	725	1,100
8	176 0	58 0	1 74	615	4 1	750	1 200
9	173 5	68 0	1 83	650	4 7	850	1 200
$\bar{x}$	178 6	65 3	1 84	659	4 9	716	1 056
S D	±7 6	±7 5	±0 42	±102	±0 59	±33	±193

of THb on weight (W) for the whole material was THb = 9 8 W − 36 0 (SD = ± 93 g, 16 5 per cent of the mean r = 0 69) The lowest residual standard deviation in per cent of the mean, for regression of THb on the antropometric parameters presented here, was for $D_{L_{CO}}$ at heart rate 120 or more (see below) 11 0 %, then height 11 1 %, and vital capacity 11 4 %, but there were significant correlations between THb and all other parameters studied

Blood volume was consequently also significantly correlated to all these parameters the lowest residual SD was on BSA, 9 % of the mean

Heart volume (HV), ml, in supine, averaged 567 ml, SD = ± 68 ml for the women and 716 ml, SD = ± 33 ml for the men HV was significantly correlated to all antropometric parameters studied The highest correlation coefficient was found for the regression of HV on the rate of work at a heart rate of 170 beats/min HV = 0 339 W_{170} + 360 (SD = ± 67, r = 0 82), then followed body weight (r = 0 71) and vital capacity

transport capacity in 9 ordinarily trained women and 9 ordinarily trained men

VC, l, BTPS	TC, l BTPS	FRC, l BTPS	Mid. cap, l, BTPS	$FEV_{1\,0}$ l, BTPS	$D_{L_{CO}}$, max ml/min/mmHg	$D_{L_{CO}}$, >120 ml min/mmHg
3 24	4 25	2 17	2 43	3 25	32 9	28.4
3 36	4 64	2 33	2 61	3 41	28.8	26 1
3 83	4 59	2 22	2 47	3 41	31 5	28.3
3 22	4 16	1 78	2 04	2 97	27 1	26 5
3 99	4 86	1 92	2 14	3 91	35 8	35 6
4 05	5 26	2 45	2 67	3 74	38 6	36 6
3 59	4 51	2 02	2 23	3 19	29 0	28 9
4 23	5 49	2 74	3 05	4 02	35 3	33 0
3 31	4 96	2 64	2 92	2 81	33 6	32 5
3 65	4 75	2 25	2.51	3 41	32 5	30 7
±0 39	±0 44	±0 32	±0 34	±0 41	±3 8	±3 9
4 66	5 77	2 51	3 03	3 96	31 0	30 8
5 43	7 70	4 15	4 46	4 68	45 5	43 0
5 37	7 10	3 85	4 10	4 68	65 0	54 0
4 27	5 59	2 75	2 97	4 18	36 5	34 9
5 83	7 31	3 29	3 58	5 12	48 6	40 7
4 86	7 21	3 86	4 03	3 41	48 5	44 4
5 22	7 29	3 73	4 16	5 06	42 8	40 7
5 26	6 73	3 67	3 94	5 23	42 3	40 9
4 98	6 39	2 90	3.23	3 96	51 8	46.7
5 10	6 79	3 41	3 72	4 48	45 8	41 8
±0 46	±0 73	±0 57	±0 54	±0 63	±9 7	±2 1

($r = 0\,72$) The regression of HV on THb was HV = 0 632 THb + 284, SD = ± 85, $r = 0\,69$

Rate of work at a heart rate of 170, (W_{170}) kpm min W_{170} averaged 608 kpm/min SD = ± 120 kpm/min for the women and 1056 kpm/min, SD = ± 193 for the men W_{170} was significantly correlated to all antropometric parameters studied The highest correlation coefficient and lowest residual SD was between W_{170} and vital capacity, $r = 0\,85$ heart volume, $r = 0\,82$ and THb, $r = 0\,77$ in that order The regression equation of W_{170} on THb was W_{170} = 1 71 THb — 135 and SD = ± 182, on HV the corresponding equation was W_{170} = 2 00 HV — 450 ($r = 0\,82$ and SD = ± 163 kpm/min)

Stat

for

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TABLE II $D_{L_{CO}} \geq 120$, ml STPD · min⁻¹ · mmHg⁻¹, related to height, cm, weight, kg (weight) $^{2}/_{3}$, BSA, m², THb, g, blood volume, l, heart volume, ml, W_{170} kpm/min VC, l BTPS, TC, l BTPS, FRC, l BTPS, Midcapacity, l BTPS, (Mid cap $^{2}/_{3}$), $FEV_{1\,0}$, l BTPS in 18 healthy, young, ordinarily trained men and women Mean value and ± S D 36 2 ± 7 8 ml min⁻¹, mmHg⁻¹ or ± 21 6 per cent of the mean

	Independent variable	Regression equation	Residual S D Units	Residual S D % of mean	b/sb	r
Body dimensions	Height	y=0 632 x —73	5 60	14 4	4 09	0 716***
	Weight	y=0 410 x +11	7 11	19 6	2 09	0 462*
	(Weight) $^{2}/_{3}$	y=1 53 x + 2 7	7 13	19 6	2 06	0 459*
	BSA	y=28 8 x − 14	6 30	17 4	3 16	0 620**
Circulatory dimensions	THb	y=0 054 x + 5 5	3 92	10 7	7 2	0 873***
	BV	y=7 56 x + 2 1	5 78	16 0	3 9	0 691**
	HV	y=0 039 x +11 4	6 60	18 2	2 8	0 569*
	W_{170}	y=0 020 x +19 3	5 50	15 2	4 3	0 729***
Pulmonary dimensions	VC	y=7 52 x + 3 3	4 54	12 5	5 8	0 824***
	TC	y=5 53 x + 4 3	4 20	11 6	6 5	0 852***
	FRC	y=8 48 x +12 2	4 65	12 8	5 6	0 815***
	MC	y=8 12 x +10 9	4 86	13 5	5 3	0 796***
	(MC) $^{2}/_{3}$	y−17 6 x − 1 4	4 87	13 5	5 2	0 795**
	$FEV_{1\,0}$	y= 6 6 x +10 1	6 17	17 0	3 3	0 640***

of the predicted normal value for the women and 94 per cent for the men *Total lung capacity*, (TC), averaged 4 75 l, BTPS, SD = ± 0 73 for the men, all within the normal range of variation when related to the above normal values

Functional residual capacity, (FRC), averaged 2 25 l, BTPS for women, SD = ± 0 32 and 3 41 ± 0 57 for the men All subjects had values normal in relation to predicted values Both FRC and residual volume, (RV) was normal in relation to TC in all subjects

Dynamic spirometry Forced expiratory volume in one second, $FEV_{1\,0}$ averaged 3 41, SD = ± 0 41 l, BTPS for women and 4 48, SD = ± 0 63 l, BTPS for men $FEV_{1.0}$ was normal in all subjects Also $FEV_{1\,0}$ was significantly correlated to all antropometric parameters studied The highest correlation coefficient was on vital capacity

Comment on material The material used for the present study consists, as judged from the presented anthropometric data, of healthy young men and women, ordinarily untrained with values for total hemoglobin, heart volume, working capacity, static and dynamic lung volumes which for all subjects fall within the normal ranges of variation

Data obtained during the determination of $D_{L_{CO}}$ are available on request, and will be published separately

Heart rate during measurement of $D_{L_{CO}}$ The highest individual values varied between 154 beats per min (male subjects, no 3) and 198 beats/min (male subjects, no 9) The

TABLE III Relationship between $DL_{CO \geq 120}$ and a number of measurements of circulatory and pulmonary dimensions after elimination of body size in nine healthy untrained men and nine healthy untrained women expressed with a multiple regression equation

$DL_{CO} = a \cdot H + b \cdot W + c \cdot x + d$, H=height, cm, W=weight, kg, n=18 Mean value ± S D for $DL_{CO \geq 120} = 36.2 \pm 7.8$ ml min^{-1} $mmHg^{-1}$ or 21 6 per cent of the mean Dimensions of dependent parameter as in table II

Parameter	a	b	c	d	Residual S.D Units	Residual S.D % of mean	Multiple corr coeff
Total hemoglobin	0 0138	−0 237	0 065***	11 7	±3 85	10 6	0 90
Blood volume	0 542	−0 301	4 79	−60 8	±5 51	15 2	0 77
Heart volume	0 754	−0 447	0 029	−85 8	±5 26	14 5	0 79
W_{170}	0 629*	−0 427	0 0168*	−60 6	±4 50	12 4	0 85
Vital capacity	0 344	−0 188	5 97*	−37 9	±4 54	12 5	0 85
Total lung capacity	0 1632	−0 033	4 72**	−17 3	±4 41	12 4	0 86

average highest individual value was 177 beats/min in both the female and the male groups

Ventilation, V_E, *l*, BTPS/min increased to highest individual values of 39 1 l, BTPS/min in the female group and 63 1 in the male group These values constitute only 39 5 and 41 9 per cent of the maximum voluntary ventilation with a respiratory rate of 40 beats/ [illegible]

in the female group and 2335 ml STPD in the male group

Arterial gas tensions Arterial oxygen tensions were measured in all subjects and were found to be normal The results will be reported elsewhere

Arterial carbon dioxide tension, was measured with both the Severinghaus electrode and the microequilibration technique on all occasions The electrode values were systematically higher, 2 9 mm Hg, than those obtained with the microequilibration technique ($P < 0.001$), *the SD of the differences was 3 0 mm Hg The regression of the* microequilibration values on the Severinghaus values was

$$P_{aCO_{2M}} = 1.024\ P_{aCO_{2S}} - 3.8,\ SD = \pm 2.85\ mm\ Hg,\ n = 134$$

The values obtained with the electrode were more reproducible, including only one single measurement compared with 3 pH measurements and 2 equilibrations of blood with gases of known P_{CO_2}, Holmgren (1964) For this reason they were chosen for the further analyses of the data although the microequilibration values were used simultaneously as a control. When P_{aCO_2} is discussed in the following only electrode values are *referred to unless specified otherwise The* P_{aCO_2} *at rest was on an average 38 2 mm Hg*, SD = ± 2 7 in the male group During exercise the subjects often hypoventilated as

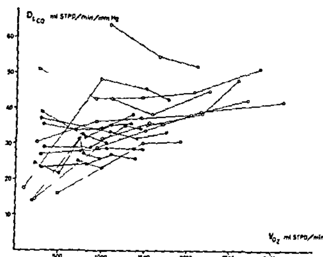


Fig 1 Relationship between diffusion capacity of the lungs for CO $D_{L_{CO}}$, ml STPD min $^{-1}$ mm Hg^{-1} (ordinates) and $\dot{V}_{O_2}$, ml STPD min $^{-1}$, (abscissa) Open symbols represent men and filled symbols women

judged by $P_{a_{CO_2}}$ — both electrode and microequilibration values, and RQ No subject did hyperventilate to any major extent during exercise in this material

pH and standard bicarbonate decreased slightly during the procedure Only in the men 1, 7, 8 and 9 did pH drop below 7 35 units

Carbon monoxide uptake, $\dot{V}_{CO}$, ml STPD, min $^{-1}$ increased approximately linearly with increasing $\dot{V}_{O_2}$ $\dot{V}_{CO} = 3\,68 \cdot 10^{-3} \cdot \dot{V}_{O_2} + 2\,52$, SD = $\pm$ 0 93, $r = 0\,93$ for men and $\dot{V}_{CO} = 4\,31 \cdot 10^{-3} \cdot \dot{V}_{O_2} + 0\,68$, SD = 0 59, $r = 0\,94$ for women The slope for the two regression lines was not significantly different The difference in intercept was highly significant ($P < 0\,001$) $\dot{V}_{CO}$ was not linearly related to alveolar ventilation, $\dot{V}_A$, l, BTPS/min as has been suggested, but more close to a hyperbola as is the case for the relationship between $\dot{V}_{O_2}$ and $\dot{V}_A$, i e there is a hyperventilation in relation to $\dot{V}_{CO}$ at higher work loads

Alveolar carbon monoxide tension $P_{A_{CO}}$, mm Hg, was also some curvilinear function of $\dot{V}_{CO}$ increasing from an average at rest of the 0 018 mm Hg to reach a level at oxygen uptakes around 1000—1500 ml STPD/min and a pressure of the order of 0 25 mm Hg

Arterial COHb and pulmonary mean capillary CO pressure, S_{CO} and $P_{\bar{c}_{CO}}$ The highest individual value for CO-saturation reached in the female group, was 13 9 (female subject No 9), the corresponding corrected back pressure (Linderholm 1957) was 0 06 mm Hg or 21 per cent of $P_{A_{CO}}$

S_{CO} reached a value of on an average 11 6 per cent (SD = $\pm$ 1 5) in the female material and 10 9 per cent (SD = $\pm$ 2 2) in the male material The *back pressure* under these conditions reached an average of 0 0486 mm Hg, at $\bar{P}_{A_{CO}}$ of 0 2408 mm Hg or 20 per cent of the alveolar P_{CO}, in the female group and 0 0401 mm Hg at a $\bar{P}_{A_{CO}}$, of

0 2804 mm Hg i e 14 per cent of $P_{A_{CO}}$ in the male group The highest individual S_{CO} in the male group was 13 3 per cent at a $P_{A_{CO}}$ of 0 3363 mm Hg or 15 per cent of $P_{A_{CO}}$

Fractional CO uptake The fractional CO uptake, CO_F *i e* $CO_F = \frac{F_{I_{CO}} - F_{E_{CO}}}{F_{I_{CO}}}$ varied as a curvilinear function of $\dot{V}_{O_2}$, decreasing from an average of 0 438 at rest to 0 314 during the highest load

Diffusing capacity of the lung, $D_{L_{CO}}$, *ml STPD min*$^{-1}$ *mm Hg*$^{-1}$

In the female group (Fig 1) $D_{L_{CO}}$ remained essentially unchanged with increasing $\dot{V}_{O_2}$ for values calculated with the aid of both electrode and microequilibration P_{CO}, [illegible] a [illegible] be

In the male material there was an increase of $D_{L_{CO}}$ on transition from rest to work in 4 subjects, a decrease in 4 and in one subject D_L remained unchanged When values obtained during work in the male group at a heart rate of 120 beats/min or more were plotted against $\dot{V}_{O_2}$, the regression line calculated with the method of least squares was $D_{L_{CO}} = 0\ 0048\ \dot{V}_{O_2} + 33$ (n = 25) The slope was not statistically significantly different from zero

For the further treatment of the observations, $D_{L_{CO}}$ was therefore assumed to remain approximately constant during exercise at heart rates of 120 beats/min or more The intraindividual variations were of an order of magnitude to be expected from the reproducibility of the method used earlier given as 7 2 per cent (Holmgren 1964)

The means of all determinations at or above a heart rate of 120 beats/min in a subject are presented in Table I together with the maximal value The mean values for $D_{L_{CO}}$ at heart rate > 120 and $D_{L_{CO\,max}}$ respectively in the female group were 30 7 ml STPD min^{-1} mm Hg^{-1} (SD = ± 3 9), and 32 5 (SD = ± 3 8) Corresponding figures for the men were 41 8 (SD = ± 2 1), and 45 8 (SD = ± 9 7) These values were, as stated under Methods, calculated with values for $P_{A_{CO}}$ obtained with the Severinghaus electrode

Relationship between $D_{L_{CO}}$ *and various anthropometric measures*

For this study two estimates of the diffusing capacity during exercise were used
1) $D_{L_{CO} > 120}$ This value was calculated as the mean of all observations during exercise, irrespective of number, at a heart rate > 120 beats/min
2) $D_{L_{CO\,max}}$ which is the highest value observed in an individual These values were then correlated with the following anthropometric parameters height, weight weight 3/4, body surface area, total hemoglobin, blood volume, heart volume, $\dot{V}_{O_2}$ $_{170}$ vital capacity, total lungcapacity functional residual capacity midcapacity midcapacity^2, $FEV_{1.0}$ Men and women were treated together Both $D_{L_{CO\,max}}$ and $D_{L_{CO} > 120}$ were used for the study but since all r-values for $D_{L_{CO} > 120}$ were higher than those for

$D_{L_{CO\,max}}$ only $D_{L_{CO_{120}}}$ estimate will be presented here (Table II and III) The relation ship between the two was $D_{L_{CO\,max}} = 1\ 235\ D_{L_{CO \geq 120}} - 5\ 59$, SD = 2 23, r = 0 976

It is reasonable to assume that the random error in $D_{L_{CO \geq 120}}$ is less than that in $D_{L_{CO\,max}}$. $D_{L_{CO \geq 120}}$ was significantly correlated to all parameters listed above The highest regression were on height (body dimension) and THb (circulatory and body dimension) The different measures of the pulmonary dimensions were all highly correlated to D_L except $FEV_{1.0}$ (Table II)

To eliminate the influence of body size, body weight and height were inserted as independent variables in a multiple regression calculation with three independent variables Measures of circulatory dimensions, THb, BV, HV and W_{170} and of pulmonary dimensions VC and TC were alternately inserted as the third independent variable The result is given in Table III Under these circumstances $D_{L_{CO}}$ is still significantly correlated to THb, W_{170} VC and TC but only regarding THb does the residual SD decrease below the lowest value for SD when only one independent variable is used for prediction

Discussion

The *material* in the present study can as stated above be characterized as young, healthy, and ordinarily untrained This judgement is based on case history, measurements of total hemoglobin, heart volume and rate of work at 170 beats per min (Sjostrand 1953)

The *methods* employed have been reported earlier, Holmgren (1964 a and b), and the reproducibility and validity of the measurements have been discussed in detail They are regarded as being satisfactory Since all computations have been performed with digital computers, IBM 1401 and 7090, after careful programming the number of calculation errors should be small

Carbon monoxide uptake was found to increase linearly with increasing $\dot{V}_{O_2}$ in both groups The level of the regression line for women was lower than that for men The present design of the $D_{L_{CO}}$ procedure, letting the subject breathe the CO-air mixture for two minutes before the actual measurement should guarantee that a steady state of the CO uptake is present, *Filley et al* (1954), *Bates, Boucot and Dormer (1955), Shephard et al* (1958) The reproducibility of $\dot{V}_{CO}$-measurements at tha same $\dot{V}_{O_2}$ but differing back pressure was found to be 7 2 per cent in the present material Holmgren (1964 a) The relationship between $\dot{V}_{CO}$ and alveolar ventilation, $\dot{V}_A$ was similar to that repeatedly demonstrated for $\dot{V}_{O_2}$ and $\dot{V}_A$ during heavy exercise i e curvilinear, with an increase in $\dot{V}_A/\dot{V}_{CO}$ at higher rates of work

The *fraction of CO removed* from the inspired gas F_{CO}, decreased curvilinearly, probably hyperbolically, with increasing $\dot{V}_{O_2}$ When plotted against ventilation $\dot{V}_E$, and alveolar ventilation, $\dot{V}_A$, the relationship was approximately linear The decrease in F_{CO} occured simultaneously with a decrease in V_D/V_T Filley *et al* (1954)

The calculation of $D_{L_{CO}}$ was performed using both electrode and microequilibration values for $P_{a_{CO_2}}$ The two measurements give values for $D_{L_{CO}}$ which are significantly different, the electrode values being 2 7 units higher The reason for selecting the electrode values is as stated above the higher reproducibility of this method

$D_{L_{CO}}$ remained essentially unchanged with increasing $\dot{V}_{O_2}$ in the female group, while in the male group there was an average increase on transition from rest to work but great

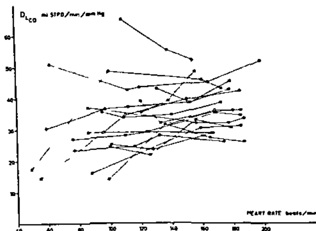


Fig 2 Relationship between $D_{L_{CO}}$ ml STPD min^{-1} mmHg^{-1}, (ordinates) and heart rate beats per minute, (abscissa) Symbols as in Fig 1

individual variations Further increases of the work load caused a slow increase in $D_{L_{CO}}$ When the male group was treated statistically the slope of this increase was not significant. These observations are consistent with those of Filley *et al* (1954) and Turino *et al* (1963) where a similar technique was used, but differ, see introduction, from most other observations using steady state or single breath technique Neither Filley *et al* (1954), nor Turino (1963) state that they have reached a maximal value Turino suggests that may be the number of experimental points are too few in the material to demonstrate a plateau for $D_{L_{CO}}$ at values for $D_{L_{CO}} > 1000$ ml/min In the present material the number of observations in an individual during exercise was three or more The observations seem to support the concept of a plateau for $D_{L_{CO}}$ during exercise, and in part of the further treatment of the data values obtained at heart rates $\geqslant$ 120 beats/min were regarded as estimates of the maximum $D_{L_{CO}}$ of the individual and the mean of these values was used as representive for $D_{L_{CO\,max}}$ cf Fig 2

The individual mean values for $D_{L_{CO}}$ thus obtained in the present study are of the same general order as those reported by Filley *et al* (1954), Bates, Boucot and Dormer, (1955) Cugell *et al* (1956), Donevan *et al* (1959), Hansson and Tabakin, (1960), Turino *et al* (1963) all using steady state measurements Donevan *et al* (1959) demonstrated a linear increase in $D_{L_{CO}}$ with increasing $\dot{V}_{O_2}$, using a steady state technique where $P_{A_{CO}}$ was calculated using dead space values calculated from a V_D/V_T relationship based on measurements of $P_{a_{CO_2}}$ published by Asmussen and Nielsen (1956), Bates *et al* (1955), and based prediction on oxygen uptake No such relationship of reasonable significance could be demonstrated with the present technique, in the present material, (Fig 3)

To get indirect information of what factors determine $D_{L_{CO}}$ within an individual, regression and multiple regression analyses before and after elimination of body size were

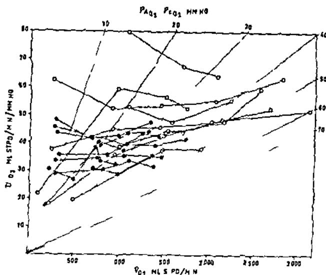


Fig 3 Relationship between diffusion capacity of the lungs for oxygen DL_{CO_2} ml STPD min mmHg⁻¹ (ordinates) and oxygen uptake V_{O_2} ml STPD/min (abscissa) Symbols as in Fig 1 Thin lines represent iso-alveolo-mean capillary oxygen partial pressure difference lines

performed using measures of body size height weight body surface area measures of some dimensions related to the size of the circulatory system total hemoglobin, heart volume blood volume and measures of the size of the lungs vital capacity total lung capacity functional residual volume, pulmonary midcapacity and forced expiratory volume in one second Since $D_{L_{CO\geq120}}$ gave systematically higher regression coefficients this estimate of $D_{L_{CO}}$ was used (Table II) $D_{L_{CO}}$ was found to be significantly correlated to all parameters in Table I As stated under results body weight)$^{2/3}$ and (Pulmonary midcapacity)$^{2/3}$ were used in an attempt to increase the correlation of D_l on weight and lung volume but no futher decrease in residual standard deviations was obtained The analyses shows in essence that the estimate of maximum $D_{L_{CO}}$ used was significantly related to measures of body size the size of the cardiovascular oxygen transport system and the size of the lungs Since all regressions were significant an attempt was performed to eliminate the influence of body size by inserting height and weight as independent variables in a multiple regression analyses It could then be shown that if body size was eliminated $D_{L_{CO}}$ was still significantly correlated to total hemoglobin a measure of the size of oxygen forwarding capacity of the circulatory system (Sjostrand 1953) to W_{170} a measure related to the working capacity of the individual and to vital capacity and to total lung capacity measures of the size of the lungs Using height weight and total hemoglobin $D_{L_{CO}}$ could be predicted with a residual standard deviation of ± 3 9 units This was however not significantly different from SD in the regression of $D_{l_{CO}}$ on THb alone (3 92 ml min⁻¹ mm Hg) These observations of a relationship between $D_{L_{CO}}$ during exercise and various anthropometric parameters verifies earlier observations by Cohn *et al* (1955) Ogilvie *et al* (1954) Bates *et*

al (1955) Hanson and Tabakin (1960) and Newman *et al* (1962), who demonstrated that D_L was related to body surface area, height and midcapacity of the lungs

Using the factor 1 23 to convert $D_{L_{CO}}$ to $D_{L_{O_2}}$ and the determined oxygen uptake, V_{O_2} the alveolo-meancapillary gradient can be calculated

$$P_{A_{O_2}} - P_{\bar{c}_{O_2}} = \frac{V_{O_2}}{\underbrace{D_{L_{CO}}\ 1\ 23}_{D_{L_{O_2}}}}$$

It is seen in *Fig* 3 that the alveolo-meancapillary oxygen gradient increases with increasing oxygen uptake reaching values between 40—50 mm Hg in the female group and 30—60 mm Hg in the male group

The present investigation thus indicates that during exercise $D_{L_{CO}}$ increases abruptly or remains unchanged during the transition from rest to work and then remains essentially unchanged from work loads above a heart rate of 120 beats/min and on Above this work level the oxygen transport is effectuated by a rising pressure head Since alveolar P_{O_2} in the present investigation remains essentially constant the increase in the pressure head is effectuated by the hyperbolic decrease in mixed venous P_{O_2} with increasing oxygen uptake

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On the Variation of $D_{L_{CO}}$ with Increasing Oxygen Uptake During Exercise in Healthy Trained Young Men and Women

By

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Abstract

Holmgren, A. *On the variation of* $D_{L_{CO}}$ *with increasing oxygen uptake during exercise in healthy trained young men and women* Acta physiol scand 1965 *65* 207—220 — $D_{L_{CO}}$ was measured [illegible]

and 47 9 units in the male group This value for $D_{L_{CO}}$ was highly correlated to a number of measures of body size, the dimensions of the cardio vascular system and the lungs. A significant correlation of $D_{L_{CO}}$ to pulmonary and circulatory dimensions remained after elimination of body size $D_{L_{CO}}$ of the present trained group was compared with that of a corresponding untrained group with covariance analyses. $D_{L_{CO}}$ in the trained group was higher than that in the untrained group This difference was matched by a difference in body size, and dimensions of the cardiovascular system and lung volumes The $D_{L_{CO}}$ of the girls was found to be lower than that of the boys This difference was larger than the difference in body size

In an earlier investigation (Freyschuss and Holmgren 1964), the variation of $D_{L_{CO}}$ in young untrained men and women during exercise with increasing load, in sitting position, was reported The results showed that $D_{L_{CO}}$ approximately reached a niveau at work loads that increased the heart rate to about 120 beats/min This measure of $D_{L_{CO}}$ was correlated to measures of body size, and the size of the cardiovascular system and

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the lungs The results were in general agreement with those reported by Riley *et al* (1954) for D_{LO_2} and with those of Filley, McIntosh and Wright (1954) and Turino *et al* (1963) for steady state D_{LCO}, but differ from those obtained *with the single breath* technique

Bates, Boucot and Dormer (1955) reported that one athlete had a higher diffusing capacity for carbon monoxide than non athletes These observations have since then been verified repeatedly (Bannister *et al* 1960, Newman, Smalley and Thomsson 1962 and Mostyn *et al* 1963)

The present investigation was undertaken to study the variation of D_{LCO} with increasing oxygen uptake, during exercise, in sitting position, with special reference to whether a level is reached or not in well trained subjects The second aspect was to compare the obtained data with those in non athletes and to study the relationship between D_{LCO} and various anthropometric variables

Methods, material and procedure

The methodology and procedure in the present investigation have been reported earlier in three reports on methods and errors involved in the determination of D_{LCO} with the steady state technique (Holmgren 1964 a, b and c) and in a report of D_{LCO} during exercise in untrained subjects (Freyschuss and Holmgren 1964)

The *material* in the present study consists of 10 healthy, well trained young women and 10 healthy, well trained young men, students from Gymnastiska Centralinstitutet Stockholm All were aquainted with the procedures form earlier experiments of the same kind

Results

The following anthropometric variables were measured in each subject, height, weight, body surface area, total hemoglobin, blood volume, heart volume, rate of work that could be performed at a heart rate of 170 beats/min, vital capacity total lung capacity, functional residual capacity, midcapacity, forced experatory volume in one second The individual data are available on request These variables were correlated against one another and a correlation matrix was prepared which is also available on request The essence of this r-matrix is that all these varibles are sigficantly correlated to one another

Total hemoglobin, (THb), *g*, averaged 497 SD ± 56 g for the women, and 768, SD ± 108 g for the men, corresponding to 0 81 and 1 07 g Hb/kg b w The regression of THb on weight for the whole material was THb = 16 4 W — 461 SD ± 87 or 25 8 % of the mean, r = 0 85 THb was significantly correlated to all the above listed variables The highest regression coefficient and lowest residual standard deviation was found for the regression of THb on total lung capacity r = 0 93 SD ± 60 g (9 5 per cent of the mean), then followed, heart volume in supine, W_{170} body surface area

Blood volume, l, was also significantly correlated to all variables listed above The lowest SD was that for the regression on heart volume in supine The mean value was 4 11 SD ± 0 46 for women and 5 51 SD ± 0 81 for men

Heart volume, (HV), ml in supine averaged 600 ml, SD ± 61 for the women and 864 ml, SD ± 131 for the men Heart volume was significantly correlated to all the above variables The highest correlation coefficient was on total hemoglobin HV = 0 96 THb + 124, r = 0 93 SD ± 65 ml or 8 1 % of the mean followed by blood volume

total lung capacity, W_{170} and body surface area The regression of HV on W_{170} kpm/min was HV = 0 401 W_{170} + 274, r = 0 83, SD ± 96 or 13 per cent of the mean

Rate of work at a heart rate of 170 beats per min W_{170} kpm/min W_{170} averaged 834 kpm/min SD ± 101 for the women and 1400 kpm/min, SD ± 238 for the men W_{170} was significantly correlated to all the above variables The highest correlation was found between W_{170} and THb, g W_{170} = 1 92 THb — 97, r = 0 91, SD ± 143 kpm/min (12 8 per cent of the mean) Then followed blood volume, vital capacity The regression of W_{170} on HV, ml was W_{170} = 1 68 HV — 113, r = 0 83, SD ± 195 (17 6 per cent of the mean)

STATIC SPIROMETRY *Vital capacity*, (VC), l BTPS, was on an average 4 15 l, SD ± 0 52 for the women and 5 85, SD ± 0 83 for the men This parameter, was the one with the highest average correlation coefficient to the above variables If other lung volumes are excluded, the highest regression was on height Vital capacity was on an average 105 per cent of predicted normal values for the women and 111 per cent for the men None had low values *Total lung capacity*, (TC), l BTPS, averaged 5 27 l, SD ± 0 74 for the women and 7 55 l, SD ± 1 02 for the men All subjects fell within the normal range of variation as judged by values predicted from the normal material

Functional residual capacity, (FRC), l BTPS, averaged 2 37 l, SD ± 0 51 for the women and 3 46, SD ± 0 53 l for the men Both FRC and residual volume were normal, absolutely, and in relation to total lung capacity

DYNAMIC SPIROMETRY *Forced expiratory volume in one second* ($FEV_{1.0}$), l BTPS averaged 3 64 SD ± 0 32 l for the women and 4 83, SD ± 0 60 for the men All subjects fell above the predicted normal value, the average per cent of predicted value being 103 per cent for the women and 113 per cent for the men Maximum volontary ventilation at a respiratory rate of 40 breaths per minute (MVV_{40}) averaged 163 5 l BTPS per min SD ± 16 9 in the male group and 122 5 l BTPS in the female groups, SD ± 11 8 The male subjects had values for MVV_{40} which were 110 per cent of the predicted values and corresponding figure for the female group was 122 per cent

Comment The above presented results for body, circulatory and pulmonary dimensions as well as the estimate of the working capacity, W_{170} all verify that the material can be classified as well trained, and healthy young men and women

RESULTS OBTAINED DURING DETERMINATION OF D_{LCO} (Individual data are available on request and will be published separately)

Heart rate rose during the experiments to highest individual values of on an average 175 5 SD ± 9 8 beats per minute in the female group and 170 4, SD ± 7 5, beats per minute in the male group Due to the variation of the order of the work loads the relationship between heart rate and $\dot{V}_{O_2}$ was accompanied by a large scatter

Ventilation ($\dot{V}_E$) l BTPS/min increased to highest individual values of 70 7 l/min in the female group and 114 5 l min in the male group These values constitute only 53 9 and 62 5 per cent respectively of the maximum volontary ventilation $\dot{V}_E$ increased approximately linearly with $\dot{V}_{O_2}$ ml STPD, according to the equations $\dot{V}_E$ = 0 030 $\dot{V}_{O_2}$ — 1 41 for the women and $\dot{V}_E$ = 0 026 $\dot{V}_{O_2}$ + 0 29 for the men These equations are almost identical with those for untrained young women and untrained young men (Freyschuss and Holmgren 1964 The increase was effectuated by an increase in both rate and tidal volume (V_T In the female group no values for V_T above 3 l BTPS were reached In the male group V_T rose to individual values as high as 4 09 l BTPS, but the majority of the values fell below 3 l

Physiological dead space, calculated with the Bohr equation (V_D for CO_2) increased linearly with V_T according to an equation $V_D = 0\ 168\ V_T + 0\ 087$, SD $\pm$ 0 086, n = 94, using the electrode P_{CO_2}, and according to the equation $V_D = 0\ 125\ V_T + 0\ 08$, SD = $\pm$ 0 11, r = 0 93 when the micro equilibration technique P_{CO_2} values were used (Holmgren, 1964 c)

Oxygen uptake, (V_{O_2}), ml STPD/min, increased to an average maximal individual value of 1968 in the female group, SD $\pm$ 307 ml/min and 3093 in the male group, SD $\pm$ 577 ml/min

Arterial gas tensions Arterial oxygen tension, ($P_{a_{O_2}}$), mm Hg, was measured at rest and during each work load $P_{a_{O_2}}$ averaged at rest, 93 0 mm Hg in the female group (range 84—101) and 91 6 mm Hg (range 72—102) in the male group During exercise, $P_{a_{O_2}}$ remained very stable in the female group but showed a tendency to decrease expecially on the highest work loads in the male group The lowest observed value in the present study was 68 mm Hg at an oxygen uptake of 3356 ml STPD/min and a $P_{a_{CO_2}}$ of 48 mm Hg (case male nr 10)

Arterial carbon dioxide tension, ($P_{a_{CO_2}}$) mm Hg, measured with the Severinghaus electrode averaged 38 6 mm Hg, SD $\pm$ 2 6 in the female group and 40 3 mm Hg, SD $\pm$ 1.2 in the male group During exercise, $P_{a_{CO_2}}$ remained essentially unchanged in the female group In the male group alveolar hypoventilation occured in subjects 1, 2 and 10 Significant alveolar hyperventilation as judged by $P_{a_{CO_2}}$ was present in case 6 load 2 A marked increase in RQ was present in case 3 load 3, case 4, load, 3, 5 load 2 and 4, 6 load 2, 7 load 2, and 10 load 4 In all these instances standard bicarbonate and pH were decreased, indicating production of non voltile acids causing an increase in V_{CO_2}

Alveolar arterial O_2 gradient (A a), mm Hg, using electrode technique for determination of $P_{a_{CO_2}}$ varied with V_{O_2}, for the whole material according to the regression equation

$$(A\ a) = 0\ 0041\ V_{O_2} + 6\ 2$$

SD $\pm$ 5 4 mm Hg or 42 per cent of the mean n — 93, r — 0 6 The error of the slope was 0 0031 and t = 1 31, i e the slope was not significant

Carbon monoxide uptake, (V_{CO}), ml STPD min^{-1}, increased approximately linearly with increasing V_{O_2} In the present material there was no significant difference between the regressions for men and women (cf Freyschuss and Holmgren 1964) and the relationship is here expressed with one regression equation for the whole material $V_{CO} = 3\ 65\ 10^{-3}\ V_{O_2} + 2\ 17$, r = 0 96 SD $\pm$ 0 79, n — 69 i e almost identical with that for untrained young men, (Freyschuss and Holmgren 1964)

Alveolar carbon monoxide tension, ($P_{A_{CO}}$), mm Hg, varied as a curvilinear function with V_{O_2}, increasing from resting values of 0 081 mm Hg to reach a level at values for V_{O_2} above 1000 ml STPD/min and a pressure of the order of 0,25 mm Hg The scatter was large due to the experimental design variation of the order of work loads and accumulation of CO in the blood

Carbon monoxide saturation of the blood ($S_{B_{CO}}$), per cent averaged 3 2 per cent, SD $\pm$ 0 5, during the determination of $D_{L_{CO}}$ at rest in the male group and 3 9 per cent, SD $\pm$ 0 6, in the female group During the repeated exposures to the carbon monoxide mixture $S_{B_{CO}}$ rose to average mximal individual values of 10 8 per cent, SD $\pm$ 1 3 in the female group and 9 3 per cent SD $\pm$ 1 7 per cent in the male group

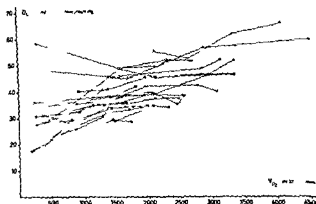


Fig 1 Relationship between diffusion capacity of the lungs for CO $D_{L_{CO}}$ ml STPD min $^{-1}$ mm Hg^{-1} (ordinates) and $\dot{V}_{O_2}$ ml STPD min (abscissa) Open symbols represent men and filled symbols women.

Pulmonary mean capillary CO pressure ($P_{\bar{c}_{CO}}$) mm Hg averaged 0 0143 at rest in the male group or 18 9 per cent of a $P_{A_{CO}}$ of 0 0757 mm Hg During exercise the highest back pressure averaged 0 0320 mm Hg SD ± 0 006 or 11 ? per cent of the average alveolar CO tension (0 2867 mm Hg) In the female group the highest CO back pressure averaged 0 0419 mm Hg SD ± 0 005 at a $\bar{P}_{A_{CO}}$ of 0 2651 mm Hg or 15 8 per cent of $P_{A_{CO}}$

Fractional CO uptake (CO_F) i e $\frac{F_{I_{CO}} - F_{E_{CO}}}{F_{I_{CO}}}$ averaged at rest 0 689 and decreased during exercise according to some curvi near function down to lowest values for CO_F in the female group of on an average 0 391 and in the male group 0 298

Diffusion capacity of the lungs ($D_{L_{CO}}$) ml STPD min $^{-1}$ mm Hg $^{-1}$ $D_{L_{CO}}$ at rest calculated using electrode values for $P_{\bar{c}_{CO}}$ averaged 23 ml STPD min $^{-1}$ mm Hg $^{-1}$ in the 5 female subjects where it was determined and 34 6 ml STPD min $^{-1}$ mm Hg in the male group In the female group there was a slight increase in $D_{L_{CO}}$ on transition from rest to work During work with increasing load there was a slow further increase The regression of $D_{L_{CO}}$ above a heart rate of 120 beats per minute on $\dot{V}_{O_2}$ was $D_{L_{CO}}$

0 0080 $\dot{V}_{O_2}$ + 26 2 r = 0 69 SD ± 6 1 n 30 (Fig 1)

The error of the slope was ± 0 0016 and t 5 0 i e the slope was highly significant 8 0 10 $^{-3}$ units ml STPD min The values at the second highest work load were however not significantly different from those at the highest work load the mean difference being 1 8 SD 0 81 units The heart rate at these work loads averaged 157 and 170 beats per minute respectively Taking into account the reproducibility of the $D_{L_{CO}}$ measurements 7 2 per cent Holmgren 1964 a the changes of $D_{L_{CO}}$ above a heart rate of 150 beats per minute are uncertain

The regression of $D_{L_{CO}}$ determinations at heart rates of ≥ 120 beats per minute on oxygen uptake in the male group was

$$D_{L_{CO}} \quad 3 95 \; 10 \quad \dot{V}_{O_2} + 28 5 \; r = 0 34 \; SD = \pm 4 5 \; n \quad 34$$

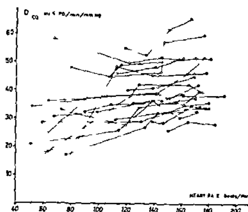


Fig 2 Relationship between $D_{L_{CO}}$ ml STPD min $^{-1}$ mm Hg $^{-1}$, (ordinates) and heart rate beats per minute, (abscissa) Symbols as in fig 1

The error of the slope was 0 0019 The slope was almost significant and 3 95 10 $^{-3}$ units ml STPD min $^{-1}$ The difference in $D_{L_{CO}}$ during exercise between the second highest and the highest load was however not significant, and the increase between these two points was on an average 1 2 mm Hg

In Fig 2 $D_{L_{CO}}$ is plotted against heart rate It is seen that that above heart rates of 120 beats per minute the changes are only slight, marked changes occur only in two subjects which because of the number studied influence the regression equation above

To decrease the random error, the mean of all measures in a subject obtained during *exercise at a heart rate of ≥ 120 beats per minute, were used as a measure of the diffusing* capacity of the lungs The mean of $D_{L_{CO \geq 120}}$ was 34 8 ml STPD/min/mm Hg, SD ± 4 0 in the female group and 47 9 units, SD ± 7 9 in the male group

The maximal values in each subjects averaged 37 2 units, SD ± 4 3 in the females and 50 6 units, SD ± 9 0 in the males The regression of the maximal $D_{L_{CO}}$ values on $D_{L_{CO \geq 120}}$ was $D_{L_{CO\,max}} = 1\,045\ D_{L_{CO \geq 120}} + 0\,7$, n = 20, r 0 98, SD ± 2 2 The slope was not significantly different from unity

To further justify the calculation of $D_{L \geq 120}$ the mean of all determinations above a heart rate of 140 beats/min, $D_{L_{CO \geq 140}}$ in the present material and in that reported by Freyschuss and Holmgren (1964) was calculated $D_{L_{CO \geq 140}}$ was significantly higher than $D_{L_{CO \geq 120}}$ ($P < 0\,001$) but the magnitude of the difference was only 1 ml/ *min/mm Hg The regression of* $D_{L_{CO \geq 120}}$ *on* $D_{L_{CO \geq 140}}$ *was* $D_{L_{CO \geq 120}} = 1\,028\ D_{L_{CO \geq 140}} - 0\,09$, r = 0 98 SD ± 1 7, n = 38 The slope was not significantly different from unity

For the analyses of the relationship between the $D_{L_{CO}}$ and various measures of body size, $D_{L_{CO \geq 120}}$ was therefore chosen as it included the largest number of measurements in each subject which would tend to decrease the random error in the estimate of $D_{L_{CO}}$

In an earlier study (Holmgren 1964c) a comparison between values for $D_{L_{CO}}$ obtained with Severinghaus electrode P_{CO_2} and microequilibration P_{CO_2} was performed The values were linearly related to each other The electrode values were 2 7 units higher than those calculated with the microequilibration technique As in the earlier

TABLE I $D_{L_{CO} \geq 120}$ ml STPD min⁻¹ mmHg⁻¹, related to, height, cm, weight kg, (weight)$^{1/3}$, body surface area BSA, m², THb, g, blood volume, BV l, heart volume HV, ml, W_{170}, [illegible] tilation at a frequency of 40 breaths/min, MVV_{40}, l BTPS/min Mean value and ±S D 41 3±9 0 ml · min⁻¹ · mmHg⁻¹ or 21 8 per cent of the mean

	Independent variable	Regression equation	Residual S D Units	% of mean	b/sb	r
Body dimensions	Height	y=0 803 x −98	6 6	16 0	4 20	0 71
	Weight	y=0 785 x −11	6 4	15 5	4 51	0 73
	(Weight)$^{1/3}$	y=3 01 x −29	6 4	15 5	4 52	0 73
	BSA	y=0 428 x −36	6 2	15 0	4 75	0 75
Circulatory dimensions	THb	y=0 0475 x +11	4 9	11 9	6 85	0 85
	BV	y=7 27 x + 7	5 7	13 8	5 52	0 79
	HV	y=0 0398 x +12	6 3	15 3	4 69	0 74
	W_{170}	y=0 0245 x +14	3 6	8 7	10 9	0 92
Pulmonary dimensions	VC	y=6 76 x + 8	5 3	12 8	6 16	0 82
	TC	y=4 91 x +10	5 7	13 8	5 46	0 79
	FRC	y=8 76 x +16	6 3	15 3	4 60	0 74
	MC	y=8 70 x +13	6 0	14 5	5 06	0 77
	(MC)$^{2/3}$	y=18.7 x + 1	6 1	14 8	4 88	0 76
	$FEV_{1 0}$	y=7 75 x + 8	7 0	16 9	3 72	0 66
	MVV_{40}	y=0 26 x + 4	6 3	15.3	4 55	0 73

study $D_{L_{CO}}$ was calculated using both the techniques for determination of P_{CO_2}. The variation of $D_{L_{CO}}$ with V_0, was the same for the microequilibration $D_{L_{CO}}$ as described above The electrode values were chosen because they were regarded as more valid and more reproducible (Holmgren 1964 c)

Relationship between $D_{L_{CO}}$ and various anthropometric measures For this study both $D_{L_{CO}\,max}$, $D_{L_{CO} \geq 140}$ and $D_{L_{CO} \geq 120}$ were used Since however all regressions on $D_{L_{CO} \geq 120}$ were higher than those on $D_{L_{CO}\,max}$ and $D_{L_{CO} \geq 140}$ only the former will be reported here As stated above $D_{L_{CO} \geq 120}$ was calculated as the mean of all determinations during exercise above a heart rate of 120 beats per minute For this study men and women were treated together The independent variables used were those listed above, height, weight, (weight)$^{1/3}$, body surface area, total hemoglobin blood volume, heart volume, W_{170} vital capacity total lung capacity, FRC, midcapacity of the lungs midcapacity$^{2/3}$, and $FEV_{1.0}$

$D_{L_{CO}}$ was as stated earlier significantly correlated to all these parameters (Table I) The highest correlation was on W_{170} If height and weight were eliminated (Table II)

TABLE II Relationship between $D_{L_{CO}\geq 120}$ and a number of measurements of circulatory with a multiple regression equation

$D_{L_{CO}} = a \quad H + b \quad W + cX + d$ where H=height, cm, W=weight, kg and X the

Dependent variable, x	Abreviation	Dimension	a	b
Total hemoglobin	THb	g	−0.197	0.134
Blood volume	BV	l	0.112	0.153
Heart volume	*HV*	*ml*	*0.211*	*0.247*
Work cap	W_{170}	kpm/min	−0.026	0.088
Vital cap	*VC*	*l*	*−0.771*	*0.315*
Total lung cap	TLC	l	−0.779	0.473

Significance *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$

$D_{L_{CO}}$ *was still significantly correlated to total hemoglobin, blood volume,* W_{170}, *vital* capacity and total lung capacity From prediction point of view, W_{170} alone gave the lowest residual standard deviation

Discussion

The *material* in the present investigation can be classfied as trained and physically fit, as judged by such variables as case history, total hemoglobin, heart volume and rate of work at 170 beats per minute, (Sjostrand 1953)

The material must be regarded as highly selected It is obvious that the young men and women who study athletics should constitute a group of people which are more devoted to physical training than others, and probably also by selection naturally more suited, for this type of activity Such a material can be used to discuss differences with untrained materials but does not necessarily elucidate the mechanisms that cause the adaption of the oxygen forwarding system when a subject is training, which would require longitudinal studies The *methodology* and procedure involved have been analyzed and discussed earlier, (Holmgren 1964 a, b and c, Freyschuss and Holmgren 1964) As in the earlier investigations all calculations were programmed and performed with IBM digital computers, 1401 and 7090

Carbon monoxide uptake increased in the present material linearly with $\dot{V}_{O_2}$ as in the earlier presented material of ordinarily trained subjects The regression lines were almost identical in the two materials

The increase in $S_{B_{CO}}$ during the experiment was moderate, and the resulting maximum back pressure constituted only 14.8 per cent of the simultaneously measured alveolar CO-pressure, a fact which tends to increase the reliability of the $D_{L_{CO}}$ measurements (Bates *et al* 1955) The experimental procedure with variation of the work loads should tend to decrease a systematic influence of the increasing $S_{B_{CO}}$ (Holmgren 1964 a)

The *work loads* employed were such as to increase heart rate to around 170 beats in each individual, thus covering light, moderate and heavy work

dimensions in 10 healthy trained young men and ten healthy trained young women expressed

third independent variable

c	Significance of c	Residual S D	SD (D_{LCO}—X)	Multiple corr coeff	r (D_{LCO}—X)
0 050	**	5 2	4 9	0 931	0 850
5 45	*	5 9	5 7	0 874	0 793
0 022		6 2	6 3	0 843	0.738
0 023	***	3 8	3 6	0 978	0 922
9 87	**	5 2	5 3	0 923	0 823
6 59	*	5 8	5 7	0 891	0 790

The relationship between V_{CO} and V_A was curvilinear also in this material and an alveolar hyperventilation in relation to V_{CO} occured during heavy work, this in accordance with observations in ordinarily trained men and women (Freyschuss and Holmgren 1964). This indicates that there is no limitation of the rate with which inspired CO molecules can be brought to the alveolar space and thus limit the fractional CO uptake, CO_F

CO_F was of the same magnitude at rest as reported earlier, (Filley *et al* 1954, Freyschuss and Holmgren 1964), and decreased to about the same extent during exercise. The relationships between F_{CO} on one hand and V_{O_2} and V_E on the other was curvilinear, *the decrease slower at higher work loads*. The decrease in F_{CO} was in this material, as in the material of Filley *et al* (1954) and Freyschuss and Holmgren (1964), due to a constancy of $D_{L_{CO}}$ during exercise.

$D_{L_{CO}}$ reached a niveau or increased only slowly during exercise with increasing load. This is in accordance with earlier observations in untrained girls and boys, using the same technique (Freyschuss and Homgren 1964), and *verifies the observations of Filley et al* (1954) and Turino *et al* (1963), but contrast to those obtained with single breath technique and the results of a number of investigators using steady state technique. For discussion see Freyschuss and Holmgren (1964). The relative niveau of $D_{L_{CO}}$ was reached at work loads increasing the heart rate to 120 beats per minute, i e at an oxygen uptake that corresponds to 40 per cent of the maximum V_{O_2} (Holmgren and Åstrand 1963). These observations are in agreement with those of Riley and coworkers (1954), who found that $D_{L_{O_2}}$ reached a plateau at an oxygen uptake of the order of 1000 ml/min.

To diminish the random error in the estimation of this niveau, the mean of all determinations obtained above a heart rate of 120 beats per minute, was calculated. Linderholm (1959) used a value for $D_{L_{CO}}$ determined at a heart rate of 140 beats per minute. *In the present material the mean of all determinations above a heart rate of 140 beats/ min was on an average one unit higher than the mean of* $D_{L_{CO}}$ above heart rates of 120 beats per minute. The maximal individual value was also recorded and was found to be 2—3 units higher than the $D_{L_{CO > 120}}$. Considering the large variability in the $D_{L_{CO}}$

TABLE III Comparison between mean values for height, cm, weight, kg, BSA, m^2, total hemo-
lung capacity, TC, l BTPS, functional residual capacity, FRC, l BTPS, pulmon
sion capacity of the lungs for CO, $D_{L_{CO} \geq 120}$, ml min^{-1} · mmHg^{-1} in eighteen un-
deviation of the mean and P probability level of the difference between untrained

Group	Height, cm	Weight, kg	BSA, m^2	THb, g	BV, l	HV, ml
Men						
Untrained	178 6	65 3	1 84	659	4 9	716
SD	7 6	7 5	0 42	102 2	0 59	32 9
Trained	178 1	71 6	1 91	768	5 5	864
SD	7 1	9 0	0 16	107 7	0 82	130 7
Diff	−0 5	+6 3	+0 70	+109	+0 6	+148
P	—	—	***	*	—	*
Women						
Untrained	168 0	57 4	1 66	471	4 1	567
SD	6 7	8 6	0 5	55 1	0 57	68 4
Trained	167 8	61 8	1 71	497	4 05	600
SD	5 1	3 8	0 08	55 9	0 49	60 9
Diff	−0 2	+4 4	+0 05	+26	+0 05	+33
P	—	—	—	—	—	—

$P < 0\ 001$ = ***, $P < 0\ 01$ = **, $P < 0\ 05$ = *

determination, using the present technique, it was regarded as important to include as many measurements as possible in the calculation of the individual mean value, even if there was a slight systematic difference between the determination at 120 beats per minute and those around 170 beats per min

The value for $D_{L_{CO} \geq 120}$ thus obtained was found to be highly correlated to measures of body size, such as height, weight and body surface area There was no further decrease in the residual SD if body weight $^3/_4$ (Kleiber 1947) was used as independent variable instead of weight $D_{L_{CO}}$ was also highly related to all static measures of the size of the lungs, — especially to vital capacity, — to the ventilatory capacity MVV$_{40}$, and to measures of the dimensions of the cardiovascular system such as THb blood volume, heart volume and its oxygen forwarding capacity as expressed by the exercise tolerance test, used, W_{170} The relationships to W_{170}, sex, to lung volume (VC) and to the size of the cardiovascular system (THb) was still significant after elimination of the influence of body size (height, weight, BSA) The highest correlation coefficient was found between $D_{L_{CO}}$ and W_{170} followed by THb and VC The highest partial correlation coefficient after elimination of W_{170} was found between $D_{L_{CO}}$ and sex followed by heart volume If VC was eliminated there was still a significant partial correlation between $D_{L_{CO}}$ on one hand and W_{170}, and THb on the other When sex was eliminated the partial

globin, THb, g, blood volyme, BV, l, $W_{1\,7\,0}$, kpm/min, Vital capacity, VC, l BTPS total ary mid capacity, MC, l BTPS, forced expiratory volume in one second, $FEV_{1.0}$, and diffu- trained young men and women and twenty trained young men and women SD is standard and trained men and women

$W_{1\,7\,0}$, kpm/min	VC, l BTPS	TC, l BTPS	FRC, l BTPS	MC, l BTPS	$FEV_{1.0}$, l BTS	$D_{L_{CO}\,>\,120}$ ml min⁻¹ mmHg⁻¹
1,056	5 10	6.79	3 41	3 72	4 48	41 8
192 8	0 46	0 73	0 57	0 54	0 63	2 1
1,400	5 85	7 55	3 46	3 81	4 83	47 9
238	0 83	1 01	0 54	0 55	0 60	7 9
+344	+0 75	+0 76	+0 05	+0 09	+0 35	+6 1
**	*	—	—	—	—	**
608	3 65	4 75	2 25	2 51	3 41	30 7
120 0	0 39	0 44	0 32	0 34	0 41	3 9
834	4 15	5.27	2 37	2 64	3 64	34 8
101 7	0 52	0 74	0 51	0 51	0 32	4 0
+226	+0 5	+0 52	+0 12	+0 13	+0 23	+4 1
***	*	—	—	—	—	*

correlation to hemoglobin concentration disappeared Elimination of the influence of total hemoglobin left only the partial correlation coefficient to W_{170} significant

For a comparison of the present material with ordinarily untrained subjects, Table III gives mean values of the present investigation simultaneosly with the mean values of corresponding data reported by Freyschuss and Holmgren (1964) for ordinarily untrained subject The means have been compared with a t-test Regarding weight and height there is no significant difference between the two materials neither for women nor for men The trained men have however a significantly larger body surface area It should be noted that the trained men are on an average 6 kg heavier then the un [illegible]

known difference in total hemoglobin, heart volume, W_{170}, and lungvolumes between trained and untrained subjects (Sjostrand 1953) The $D_{L_{CO}\,>\,170}$ is significantly higher in the trained material for both men and women

TABLE IV Comparison between the regression lines of $D_{L_{CO} \geq 120}$, ml/min/mmHg on height cm, BSA, m², total hemoglobin THb, g heart volume, HV, ml, exercise tolerance, W_{170}, kpm/min and vital capacity VC, l, for ordinarily trained boys and girls (Freyshuss and Holmgren 1964) and the trained boys and girls in the present study, and between girls and boys in the same materials The method of covariance is that of Hald (1962) The regression lines are available in table II in the present report and in that of Freyshuss and Holmgren (1964)

Grouping of *material*	*Independent variable*	Significance *difference in slope*	Weighted *mean of slopes*	Vertical *distance between lines*	Significance of *difference in level*
Trained versus untrained	Height, cm	>0.4	0.714	5.31	<0.02*
	BSA, m²	>0.2	35.75	3.06	>0.1
	THb, g	>0.5	0.0499	1.72	>0.2
	HV, ml	>0.5	0.0394	1.52	>0.4
	W_{170}, kpm/min	>0.4	0.0230	1.46	>0.3
	VC l	>0.6	7.023	0.72	>0.6
Men versus women	*Height, cm*	>0.3	*0.392*	*8.08*	<0.01**
	BSA, *m²*	>0.3	23.04	7.74	<0.01**
	THb g	>0.7	0.0490	0.83	>0.7
	HV ml	>0.9	0.0241	7.11	<0.02*
	W_{170} kpm/min	>0.5	0.0196	2.16	>0.03
	VC, l	*=0.7*	*6.265*	*2.26*	*>0.03*

In Table IV the regression lines for $D_{L_{CO} \geq 120}$ on a number of variables, height, *BSA, THb, HV,* W_{170} *and VC in the two materials were compared with covariance analyses, to see wether the regression lines differ in the two materials* The slope of the regression of $D_{L_{CO}}$ on these independent variables was not significantly different between trained and untrained subjects and a weighted mean slope was calculated With the aid of this mean slope a vertical distance between the two lines was calculated The 'trained' regression lines always lie above the 'untrained' ones but only regarding height is the vertical distance between the lines significant

The implication of this analyses is that the observed large $D_{L_{CO}}$ in the trained group is, except regarding height matched by an increase in the size of the body, of the cardiovascular system and of the lungs The present study verifies the earlier observations (Bates *et al* 1955, Bannister *et al* 1960 Newman *et al* 1962 and Mostyn *et al* 1963), that trained subjects have higher $D_{L_{CO}}$ than untrained This difference is accompanied by a corresponding difference in dimensions of body cardiovascular system and lungs It is interesting to observe that both trained and untrained subjects have the same FRC and almost the same midcapacity The larger total lung capacity in the trained subjects is the result of an increased vital capacity The linear regressions of $D_{L_{CO}}$ on VC has the same slope for trained and untrained subject but the vertical distance is 0.7 l, which is however not statistically significant

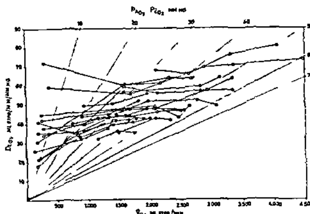


Fig 3 Relationship between diffusion capacity of the lungs for oxygen, DL_{O_2}, ml STPD min^{-1} mm Hg^{-1} (ordinates) and oxygen uptake $\dot{V}_{O_2}$, ml STPD/min (abscissa) Symbols as in fig 1 Thin lines represent isoalveolo-mean capillary oxygen partial pressure difference lines

$D_{L_{CO}}$ measured with the present technique includes the diffusing capacity of the pulmonary membrane, D_M and the diffusing capacity of the average number of red cells in the pulmonary capillaries The variables determining D_M are the area of the pulmonary surface, the thickness and the quality of the pulmonary membrane, the diffusing coefficient of CO in the membrane and the solubility of CO in the membrane The

capacity of the capillary red cells is determined by such factors as hemoglobin concentration (and thus also sex), and measures of the size of the cardiovascular system as for instance THb As it seems reasonable to assume that the number of alveoli remains unaffected by training and as FRC and MC are the same in the trained and untrained groups and a difference in MC should not necessaryly affect the area of the air-tissue or tissue blood interfaces the higher $D_{L_{CO}}$ in the trained materials is probably due to a *difference in the diffusing capacity of the pumonary capillary red cells* As the hemoglobin concentration did not differ between the trained and untrained subjects in this study the higher diffusing capacity of pulmonary capillary red cells probably reflects an increase in pulmonary capillary blood volume This view is supported by that of Mostyn *et al* (1963)

In Table IV men are compared with women There was no difference in slope for the relation between $D_{L_{CO}}$ and the above mentioned independent variables Regarding *height, BSA and heart volume there is* a significant difference in the level of the regression lines, while concerning total hemoglobin W_{170} and vital capacity the regression lines are not significantly different from one another

For a given body size and heart volume the young men have a larger $D_{L_{CO}}$ than the women *while when $D_{L_{CO}}$ is related to exercise tolerance,* W_{170} vital capacity and total hemoglobin they do not differ The partial correlation coefficient between $D_{L_{CO}}$ and sex looses its significance after elimination of hemoglobin concentration

1964) Studies in this field have recently received a new tool by the advent of the [illegible] chemical method of Falck and Hillarp (for references, see Norberg and Hamberger 19[illegible]) which permits the direct observation at cellular and subcellular levels of man[illegible] phenomena related to the effects of drugs influencing intraneuronal monoamine [illegible] The normal appearance of the sympathetic adrenergic neuron, and the intraneuronal distribution of the adrenergic transmitter, as visualized by this method has [illegible] been described and discussed in detail (Norberg and Hamberger 1964) In the present study, the effects of certain drugs known to interfere with the metabolism of the adren[illegible]ergic transmitter have been studied by this method Results with special reference to the reactions of the adrenergic cell bodies are reported in this paper The adrenergic nerve terminals in certain effector organs have also been studied, for the sake of comparison Much more large scale studies of this kind have recently been made in this laboratory, using a special iris preparation which has proved extremely suitable for this type of approach (Malmfors 1965)

Material and methods

Male albino rats (Sprague Dawley) with a body weight of 150 to 200 g were used for the experiments

The following drugs were used (when not otherwise stated they were dissolved in 0 9 per cent NaCl)

[illegible] Abbott [illegible] n mal amount [illegible] f about 5 [illegible] nylcypromine [illegible] DL-[illegible] methyl DOPA [illegible] ride (– [illegible] ydrox [illegible] bitartrate (—) α methyl NA hydrochloride (Corbasil [illegible]) hydrazine fumarate (NSD 1015 Smith & Nephew Research Ltd)

The doses of CA and reserpine were calculated as the free base The i v injections (subl[illegible] vein) were performed under light ether anaesthesia The animals were killed by bleeding [illegible] under light ether anaesthesia and dissected at once For numbers of animals and treatment see Table I—III

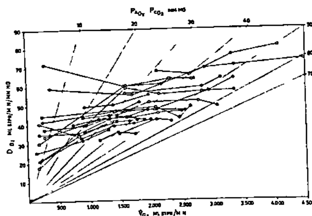
Sympathetic cell bodies from the superior cervical and stellate ganglia were studied together with their corresponding terminals in the heart and submaxillary gland Terminals in the vas deferens were also studied In some experiments, the nodose ganglia and pieces of [illegible] [illegible] ldehyde gas of op[illegible] [illegible] sectioned at 7 to 10 μ and [illegible] mounted in Entellan (Merck) for fluorescence microscopy (for deta[illegible] see Norberg and Hamberger 1964)

When [illegible] when studying the [illegible] drug the material [illegible] as in the same ba[illegible] ually several — [illegible] tical experiments for each type of treatment Each experiment included at least 2 animals Tissues were sometimes examined without knowing the treatment given to the animals

Results

A *Depletion and recovery of intraneuronal CA*

1 *Reserpine* (*Table I A and B*) Studies were performed on the depletion and of NA which occurs in the sympathetic adrenergic cell bodies (Fig 1)



$D_{L_{CO}}$ measured with the present technique includes the diffusing capacity of the pulmonary membrane, D_M and the diffusing capacity of the average number of red cells in the pulmonary capillaries The variables determining D_M are the area of the pulmonary surface, the thickness and the quality of the pulmonary membrane, the diffusing coefficient of CO in the membrane and the solubility of CO in the membrane The diffusing capacity of the capillary red cells is determined by the reaction rate between

et al (1963)

lines are not significantly different from one another

For a given body size and heart volume the young men have a larger $D_{L_{CO}}$ than the women, while when $D_{L_{CO}}$ is related to exercise tolerance, W_{170}, vital capacity and total hemoglobin they do not differ The partial correlation coefficient between $D_{L_{CO}}$ and sex looses its significance after elimination of hemoglobin concentration

Table IV thus shows that if one wants a single parameter for prediction of $D_{L_{CO}}$ in materials composed of trained and untrained young men and women, total hemoglobin vital capacity or $W_{1\,0}$ should be suitable, a fact which is of practical importance since these variable have the highest correlation coefficients on $D_{L_{CO}}$ and lowest residual standard deviations, and are relatively simple to measure

In Fig 3 $D_{L_{CO}}$ is converted to $D_{L_{O_2}}$ by multiplying it with 1 23 The oblique lines thus represent iso-alveolo mean capillary oxygen differences The necessary pressure head at the highest oxygen uptake in each individual varies between 40 and 63 mm Hg for the men and 35 and 55 for the women

The relationship between $(P_{A_{O_2}} - P_{\bar{c}_{O_2}})$ *and* V_{O_2} *could be approximated to be linear* The regression equation for the men was

$$\Delta P = 0\,014\ V_{O_2} + 6\,9,\ S\,D = \pm 6\,42\ n = 45$$

and for the women

$$\Delta P = 0\,020\ V_{O_2} + 5\,3,\ S\,D = \pm 4\,0\ n = 40$$

The difference in slope between the sexes was significant The pressure head thus increases steeper with increasing oxygen uptake for the women than for the men This is explained by the hyperbolic relationship between $D_{L_{O_2}}$ and oxygen uptake when $D_{L_{O_2}}$ reaches a niveau at a higher value for the men than for the women

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Drug-Induced Changes in Monoamine Levels in the Sympathetic Adrenergic Ganglion Cells and Terminals

A histochemical study

By

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Abstract

Norberg, K.-A. *Drug induced changes in monoamine levels in the sympathetic adrenergic ganglion cells and terminals. A histochemical study.* Acta physiol. scand. 1965. *65.* 221—234. — The cell bodies of

New concepts regarding the mechanisms of uptake, storage and release of CA[1] in adrenergic nerves, based on extensive biochemical studies, have been forwarded in recent years (see Euler 1962, Axelrod 1964, Carlsson 1964a, b, Kopin 1964, Stjarne

[1] Abbreviations used: DA = dopamine, NA = noradrenaline, CA = catecholamine(s), 5-HT = 5-hydroxytryptamine, 5-HTP = 5-hydroxytryptophan, DOPA = 3,4-dihydroxyphenylalanine, α-MMT = α-methyl-m-tyrosine, MAO = monoamine oxidase

1964) Studies in this field have recently received a new tool by the advent of the histochemical method of Falck and Hillarp (for references, see Norberg and Hamberger 1964) which permits the direct observation at cellular and subcellular levels of many of the phenomena related to the effects of drugs influencing intraneuronal monoamine levels The normal appearance of the sympathetic adrenergic neuron, and the intraneuronal distribution of the adrenergic transmitter, as visualized by this method, has recently been described and discussed in detail (Norberg and Hamberger 1964) In the present study, the effects of certain drugs known to interfere with the metabolism of the adrenergic transmitter have been studied by this method Results with special reference to the reactions of the adrenergic cell bodies are reported in this paper The adrenergic nerve terminals in certain effector organs have also been studied, for the sake of comparison Much more large scale studies of this kind have recently been made in this laboratory, using a special iris preparation which has proved extremely suitable for this type of approach (Malmfors 1965)

Material and methods

Male albino rats (Sprague Dawley) with a body weight of 150 to 200 g were used for the experiments

The following drugs were used (when not otherwise stated they were dissolved in 0 9 per cent NaCl) [illegible]

[illegible] sine (Sigma) DL-α [illegible] L-α methyl DOPA [illegible] drochloride, (—) NA [illegible] metahydroxy benzyl [illegible] bl ngual [illegible]

Sympathetic cell bodies from the superior cervical and stellate ganglia were studied together with their corresponding terminals in the heart and submaxillary gland Terminals in the vas deferens were also studied In some experiments the nodose ganglia and pieces of intestine [illegible] the sake of comparison [illegible] de gas of optimum [illegible] ed at 7 to 10 μ and [illegible] Norberg and Hamberger 1964)

When comparing the effects of different doses of the same drug or when studying the time [illegible] the material in [illegible] e batch [illegible] — iden[illegible] animals [illegible] tical experiments for each type of treatment Each experiment [illegible] Tissues were sometimes examined without knowing the treatment given to the animals

Results

A *Depletion and recovery of intraneuronal CA*

1 *Reserpine (Table I A and B)* Studies were performed on the depletion and recovery of NA which occurs in the sympathetic adrenergic cell bodies Fig 1) and terminals

TABLE I

Drug dose (mg/kg b w) and time before killing	Cell bodies	Terminals	Drug, dose (mg/kg b w) and time before killing	Cell bodies	Terminals
A Reserpine 5—10, i p			B Reserpine 12 h		
1 h (4+4)	±	+	0 1 (4)	2 to 3+	+
3 h (4+4)	0	0	0 5 (4)	—	0
6 h (4+4)	0	0	1 (4)	(+)	0
12 h (4+4)	0 to (+)	0	5 (4)	0	0
24 h (6+6)	+	0	10 (4)	0	0
48 h (6+6)	2+	0 to (±)			
96 h (6+6)	3+	± to 2+			
C. α MMT 2×400, i p at interval of 6 h After the 2nd dose			D m Tyrosine 3 × 400, i p at interval of 2 h After last dose		
1 h (4)	(+)	(+)	2 h (4)	(+)	(+)
3 h (4)	(+)	(+)	4 h (4)	(+)	(±)
6 h (4)	(+)	(+)	6 h (4)	±	+
12 h (4)	(+) to +	(+)	8 h (4)	+ to 2+	+ to 2+
24 h (4)	+	(+) to +			
48 h (4)	2+	+			
96 h (4)	2 to 3+	2+			

Symbols used 3+ *(untreated control)* 2+, +, (±), 0 *(no specific fluorescence)*

The symbols illustrate the general course and relationships within each type of treatment and represent average intensities among ganglion cells and terminals estimated from a large number of observations For details see text The intensities in cell bodies and terminals are not directly comparable Number of rats within brackets

after

with

a very

mostly still visible After 3 hrs no fluorescence of the specific type was found either in the cell bodies or terminals A weak fluorescence was found again in a few cells at 12 hrs and in the majority of the cell bodies 24 hrs after administration Almost normal fluorescence was found in the cell bodies after 48 hrs The terminals on the other hand, usually showed no, or at most a very weak fluorescence at 48 hrs Not until at 96 hrs was there always found a distinct or even very marked recovery The minimal dose which almost prevented a specific fluorescence from appearing in the cell bodies at 12 hrs — when no or only very faint recovery (in a few cells) was found after a high dose — was 1 mg/kg With the terminals as little as 0 1 mg/kg produced a clear reduction in fluorescence as compared to untreated animals and no fluorescence at all was found after 0 5 mg/kg

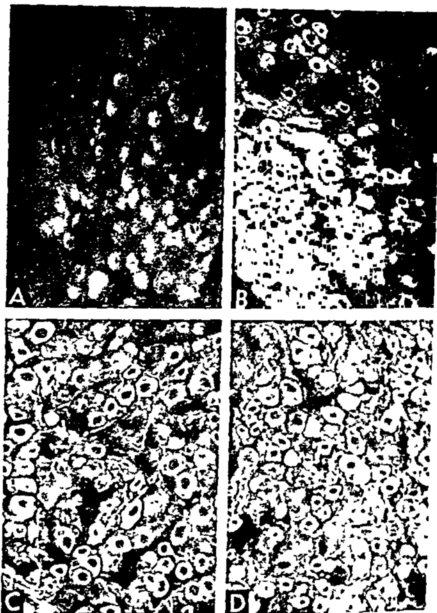


Fig 1 Sup. cervical ganglion rat Depletion and recovery of CA after reserpine 10 mg kg i p × 180 30 sec exp time

a) 6 h Only faint apparently unspecific fluorescence visible with the same intensity in both nuclei and cytoplasm

b) 48 h Very marked recovery with variation in intensity between cell bodies

c) 96 h Intensity about normal in most cell bodies

d) Untreated control

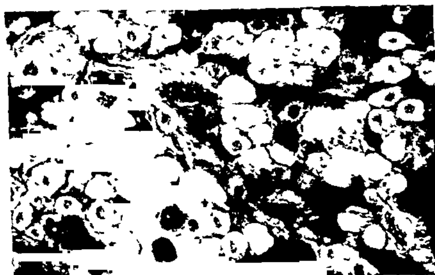


Fig 2 Stellate gangl on rat MO 911 100 mg kg 4 5 h
The variation in intensity between cell bodies is even more pronounced than normal. Some of the cells with low fluorescence intensity belong to non adrenergic neurons × 290

The fluorescence reappeared first in a small number of cells so that the variation in fluorescence intensity which is characteristic of normal ganglion cells was found also from the beginning of recovery after a complete depletion All terminals belonging to the same system e g the acinar terminals of the submaxillary gland were found to react more or less uniformly and simultaneously Differences — although not very marked — were found, however, between the terminals belonging to different systems The vasomotor terminals in the submaxillary gland and the terminals to the muscle layers in the vas deferens, for instance, generally showed a slower depletion and quicker recovery than the nerves innervating the acini

2 *a MMT* (*Table I C*) Practically no specific fluorescence was found in the cell bodies or terminals 1 to 6 hrs after the second dose of *a* MMT (400 mg/kg × 2 6 hrs interval) In the cell bodies a slight recovery seemed to have occurred at 12 hrs and a distinct to very marked recovery was found at 24 hrs while the terminals showed a very marked recovery first at 48 hrs The ganglion cells appeared almost normal at this time As with reserpine a certain degree of variation was found in the intensity of the cell bodies even from the very first signs of recovery Not even after 96 hrs was the intensity entirely normal in either part of the neuron

3 *m Tyrosine* (*Table I D*) DL-*m* Tyrosine given in 3 doses of 400 mg/kg at intervals of 2 hrs resulted in faint or no specific fluorescence in the cell bodies and terminals 2 to 4 hrs after the last dose Sure signs of recovery were found simultaneously in both cell bodies and terminals 6 to 8 hrs after the last dose

B *MAO inhibitors*

A very slight increase in fluoresence intensity as compared with the ganglia from untreated animals was observed in the cell bodies after nialamide 100 to 500 mg/kg i p 4 to 6 hrs 10 rats while the effects of pargyline (MO 911 100 to 400 mg/kg i p ,

TABLE II

Drug dose (mg/kg b w) and time before killing	Cell bodies	Terminals	Remarks
L-DOPA 50 s c 1/2—2 h (6)	no or slight increase	no increase	
Nialamide 100—500 i.p 5—7 h + L-DOPA 50—100 s c. 1/2—2 h (12)	marked increase	slight increase	
DL-5-HTP 80 s c. 1/2—1 h (4)	no increase	no increase	No yellow fluorescence
DA 50 i.p 1—4 h (6)	no increase	no increase	
NA 0 05 i.v 1/4 h (4)	no increase	no increase	
Nialamide 100—500 i p 5—7 h DA 50 i.p 1 h (4)	marked increase	slight increase	
Nialamide 100 i p 5 1/2 h + NA 0 05 i.v 1/4 h (4)	slight increase	slight increase	
α methyl DOPA 400 i p or s c 1 h (6)	very marked increase	no increase	
2 h (6)	marked increase	no increase	Lesser variation in intensity than normal between the cell bodies
4 h (6)	slight increase	no increase	
8 h (6)	no increase	no increase	

The intensities of fluorescence are given relative to tissues from untreated controls in the same experiments. For details, see text. Number of rats within brackets

4 to 6 hrs, 10 rats, Fig 2) and pheniprazine (JB 516, 10 to 40 mg/kg i p 6 hrs, 8 rats) were more pronounced After the latter two drugs, a fairly large proportion of the ganglion cells showed a very strong fluorescence in the perinuclear cytoplasm while others were of only medium fluorescence intensity As a result, even more pronounced differences than normal in fluorescence intensity were found between the cell bodies Tranylcypromine (10 mg/kg i p 4 to 5 hrs, 4 rats) produced no or only a slight increase in the fluorescence of the cell bodies The nerve terminals showed a moderate increase in fluorescence intensity after all four drugs

C. Intraneuronal uptake and accumulation of monoamines and their precursors

1 *Catecholamines and L-DOPA (Table II and III* No or only a very slight increase in fluorescence intensity as compared with the ganglia from untreated animals, was found in the adrenergic cell bodies and terminals 1/2 to 2 hrs after L-DOPA (50 mg/kg s c , 1 to 4 hrs after DA 50 mg/kg i p and 1/4 hr after NA 0 05 mg/kg i.v) If on the other hand the animals were pretreated with nialamide 100 to 500 mg/kg) 5 to 7 hrs before the amines or the L-DOPA the increase in intensity was considerable

TABLE III

Drug dose (mg/kg b.w.) and time before killing	Cell bodies	Terminals	Remarks
Reserpine 5 i.p. 9 h + Nialamide 100 i.p. 5 h (4)	(+)	0 to (+)	
Reserpine 5 i.p. 6 h + L-DOPA 40 s.c. 1–2 h (1)	0 to (+)	0 to (+)	
Reserpine 5–10 i.p. 5–9 h + DL-5 HTP 80 s.c. $^1/_2$–1 h (8)	0 to (+)	0 to (+)	No yellow fluorescence
Reserpine 5–10 i.p. 3–24 h + Nialamide 100–500 i.p. 5 h + L-DOPA 10–100 i.p. or s.c. $^1/_4$–3 h (26)	2 to 3	2 +	Lesser variation in intensity than normal between the cell bodies
Reserpine 5 i.p. 9 h + Nialamide 100 i.p. 5 h + L-DOPA 50 s.c. after ligation of abdominal aorta 1 h (3)	3 +	2 +	*Lesser variations* in intensity than normal between the cell bodies
Reserpine 5 i.p. 9 h + Nialamide 100 i.p. 5 h + NSD 1015 100 i.p. 2 h + L-DOPA 50 s.c. 1 h (4)	+	0	Lesser variations in intensity than normal between the cell bodies
Reserpine 5 i.p. 9 h + Nialamide 100 i.p. 5 h + DL-5 HTP 80–100 s.c. 1 h (8)	(+)	0 to (+)	No yellow fluorescence
Reserpine 10 i.p. 16–20 h + Nialamide 100 i.p. 4–5 h + DA 1–10 i.p. or s.c. 1–2 h (8)	2 +	2 +	
Reserpine 5–10 i.p. 9–14 h + Nialamide 100–500 i.p. 4–5 h + NA 0.05 i.v. $^1/_4$–$^1/_2$ h (6) or NA 0.25–2.5 i.p. 1 h (8)	2 +	2 +	
Reserpine 5–10 i.p. 4–6 h + α-methyl DOPA 5–100 i.p. or s.c. 1–2 h (30)	+ to 3 +	0 to (+)	
Reserpine 5 i.p. 9 h + α-methyl NA 0.2 i.v. $^1/_4$ h + α-methyl NA 0.1 i.v. $^1/_4$ h (10)	2 + to 3 +	3 +	Lesser variation in intensity than normal between the cell bodies

Symbols used, see Table I. Number of rats within brackets.

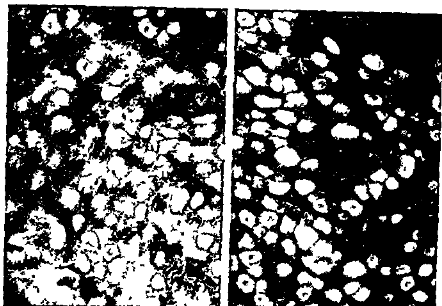


*Fig 3 (left) Stellate ganglion rat reserpine 5 mg/kg 9 h + nialamide 100 mg/kg 5 h + L-*DOPA 50 mg kg 1 h Moderate fluorescence intensity in most cell bodies in this case with some variation in intensity although not as pronounced as in the ganglia from untreated controls. × 230

Fig 4 right Sup cervical ganglion rat reserpine 10 mg kg 5 h + *a* methyl DOPA 25 mg kg 2 h Moderate and even fluorescence intensity in the cell bodies including the nuclei × 230

The administration of *a* methyl DOPA (400 mg kg s c or i p induced within 1 hr a very high over all fluorescence intensity in the background and in the ganglion cells including the nuclei After 2 hrs the over all intensity was less prominent, and certain cells which are undoubtedly identical with those lacking any specific fluorescence in the untreated controls had a considerably lower intensity than the rest Some degree of fluorescence was still observed in the nuclei After 4 hrs the fluorescence intensity of the cell bodies was somewhat higher than normal and after 8 hrs no clear difference could be observed between the ganglia from normal and treated animals

In reserpine treated animals the administration of L-DOPA 40 mg kg s c 1 to 2 hrs caused no or only a very faint fluorescence in the cell bodies and terminals Pretreatment of the animals with reserpine and nialamide 100 to 500 mg kg 5 hrs) before the L-DOPA produced on the other hand a quite high fluorescence intensity in both cell bodies Fig 3 and terminals Some degree of fluorescence — although of lower intensity than in the cytoplasm — was present also in the nuclei while certain cells which are undoubtedly identical with the cells lacking fluorescence in untreated controls see Norberg and Hamberger 1964) showed practically no fluorescence In contrast to normal animals the intensity of fluorescence varied very little between the different ganglion cells The administration of nialamide only (100 mg kg 5 hrs to animals pretreated with reserpine produced a weak green

fluorescence in the cell bodies and terminals. When the aorta was tied below the diaphragm before the administration of L-DOPA to minimize the extraneuronal decarboxylation of DOPA an intense even fluorescence was still found in the peri nuclear cytoplasm and some fluorescence also in the nuclei After L-DOPA preceded by reserpine, nialamide and the DOPA decarboxylase inhibitor NSD 1015 the terminals showed no specific fluorescence, while the cell bodies also in this case showed increase in fluorescence intensity as compared with the controls The administration of DA or NA to *animals pretreated with reserpine and nialamide* also resulted in an increased fluorescence in the cell bodies and terminals, without the variation normally observed among cell bodies

The administration of α methyl DOPA (5 to 100 mg/kg i p or s c , 1 to 2 hrs) to animals pretreated with reserpine resulted in a fluorescence of rather even intensity in the cell bodies (Fig 4), while no or only a very faint fluorescence was found in the terminals The administration to reserpine pretreated animals of α methyl NA in two doses, (0 2 and 0 1 mg/kg i v , 15 and 10 min respectively) before killing resulted in a strong fluorescence in the cell bodies, with a less pronounced variation than normal The terminals in the effector organs showed about the same intensity as in untreated animals

No accumulation was found after nialamide plus L-DOPA DA NA or α methyl NA in either sensory (nodose ganglion cells) or other nonadrenergic ganglion cells (non fluorescent cells in the sympathetic ganglia and intramural ganglion cells in the intestine)

2 *5 HTP (Table II and III)* None of the yellow fluorescence typical of 5 HT or 5-HTP was observed in the cell bodies or terminals after the administration of DL-5-HTP (80 to 100 mg/kg), either in normal animals or after pretreatment with reserpine or reserpine plus nialamide In the latter case there was found a faint greenish fluorescence of the same intensity and colour as after reserpine plus nialamide alone

Discussion

The chemical basis and specificity of the fluorescence reaction have been discussed in recent papers (see Dahlström and Fuxe 1964 Norberg and Hamberger 1964), and permit the conclusion that normally a green or yellow green fluorescence in peripheral neurons — in agreement with biochemical data — is caused by the presence of NA (*cf* Norberg and Hamberger 1964) Other compounds, however, such as DOPA and the α methylated amine analogues which give the same type of fluorescence, are of importance under the experimental conditions of the present investigation

The fluorescence method has a very high sensitivity but as yet only semiquantitative estimations can be made From calculations of the concentration of CA based on both fluorescence microscopy and biochemical data and when the respective thickness of [illegible]

berger 1964)

Depletion and recovery of intraneuronal CA It can be inferred from the present results that reserpine causes about the same rapid depletion of intraneuronal CA — within 3 hrs — in the cell bodies as in the terminals This can be compared with biochemical data indicating that NA depletion in the superior cervical ganglia of the rabbit is

maximal within 4 hrs (Muscholl and Vogt 1958 Reinert 1963) The minimal dose of reserpine necessary to produce a depletion of NA was found to be 5 to 10 times higher for the cell bodies than for the terminals The same is true of the central CA neurons (*Dahlström and Fuxe 1964*) *Differences of this kind have been found also by biochemical* determinations of NA in the superior cervical ganglia, iris and nictitating membrane in cat (Andén and Henning 1965, personal communication) The reason for these differences is unknown but it must be remembered that the ratio surface area/protoplasmic volume is much higher for the varicosities of the terminals than for the cell bodies (about 20 1 if the cell body is a sphere with a diameter of 30 μ and a varicosity a cylinder with the dimensions 1 × 0 5 μ) This can be of importance with a compound like reserpine, which seems to penetrate the neuronal membrane by virtue of its lipophilic character rather than by any active transport mechanism (Carlsson and Waldeck 1965) *The depletion following α MMT and m tyrosine was also rapid in both cell* bodies and terminals, although the fluorescence in the former never was entirely absent after these drugs It is very difficult however, to get a complete depletion of NA even with repeated large doses (Andén 1964a) There is little doubt that the rapid depletion of the NA in the sympathetic adrenergic neuron following administration of the DOPA analogues *α* MMT and *m* tyrosine is effected in fact by their amines formed by intraneuronal decarboxylation and subsequent *β* hydroxylation (Andén 1964a, Carlsson 1964a Shore, Busfield and Alpers 1964) The amines thus formed seem to displace the endogenous NA in the storage granules

The first signs of recovery after reserpine or *α* MMT treatment were observed much earlier in the cell bodies than in the terminals After *m* tyrosine on the other hand recovery was much more rapid and occurred simultaneously in both cell bodies and terminals The reason for this latter observation is probably that the amines formed from *m* tyrosine are rapidly degraded by the MAO The long lasting effect of *α* MMT *may be due at least partly to the circumstances that its amine metabolites, α* methyl *m* tyramine and metaraminol are not readily attacked by MAO and that the metaraminol seems to be firmly bound to the storage granules (Carlsson 1964a Andén 1964a) — From the very beginning of recovery after depletion by reserpine or *α* MMT the cell bodies showed the normal variation in fluorescence intensity This is in agreement with the finding that the adrenergic ganglion cells have different rates of formation and or down transport of amine storage granules (Dahlström 1965) The heterogeneity of the ganglion cells in this respect has been discussed in a previous paper (Norberg and Hamberger 1964) In all probability the fluorescence observed in recovery after reserpine treatment is due almost exclusively to the presence of NA since its precursors cannot normally be [illegible] (Norberg and Hamberger 1964) [illegible] gest some recovery of NA in [illegible] The slow recovery in the terminals in rats is in agreement with the biochemical data for reserpine (*Andén, Magnusson and Waldeck 1964*) *and for α* MMT (Hess *et al* 1961 Andén 1964a) and with histochemical studies on the iris terminals (Malmfors 1965

It has now been well established that the amine storage granules in both central and peripheral adrenergic neurons are formed at a high rate in the cell bodies and then transported down to the terminals Dahlström 1965 Dahlström Fuxe and Hillarp 1965) There is also *little doubt that the specific action of reserpine is to produce a very* long lasting blockage of the uptake storage mechanism of the amine granules (for references see Dahlström Fuxe and Hillarp 1965) The most logical explanation of

the rapid recovery in the cell bodies is consequently that newly formed storage granules containing NA begin to accumulate in the cells at a time when the granules in the terminals are still blocked and cannot store any amines The fact that a similar difference was found in recovery after the administration of α MMT, which acts in a quite different way from reserpine (see above), gives further support to this view (*cf* Dahlstrom, Fuxe and Hillarp 1965) — Some observations on depletion and recovery of NA were briefly reported by Falck (1962)

In agreement with findings in the central nervous system (Carlsson, Falck and Hillarp 1962, Fuxe 1965) certain systems of adrenergic terminals (in the vas deferens and blood vessels) showed a lower sensitivity to reserpine and also a quicker recovery than other terminals (around the acini of the submaxillary gland) The explanation for this is unknown, but possibility differences in impulse activity may be of importance

The effect of MAO inhibitors The present experiments show that the two chemically different MAO inhibitors pargyline (MO 911) and pheniprazine (JB 516) give markedly higher levels of a primary CA, presumably NA, than is normally present in the fluorescent cell bodies of the rat superior cervical ganglion, indicating that the increased NA content is localized predominantly in these The ganglion cells were found to react largely as two different populations, one showing a more or less pronounced amine increase and the other no or only a slight increase The ganglion cells of the sympathetic ganglia differ markedly in respect of MAO activity (as judged from the results obtained by histochemical methods, Koelle and Valk 1954, Norberg unpublished data) It is therefore possible that MAO plays a much more prominent role in CA metabolism in some cells than in others This could explain the present observations

Uptake and accumulation of different CA and their precursors The present results confirm [illegible]

types of peripheral nerve cells such as the sensory (*e g* the nodose ganglion cells), and parasympathetic (*e g* the intramural ganglion cells in the intestine, the submaxillary ganglion cells) nerve cells and certain cells in the sympathetic ganglia of the rat, which are in all probability identical with those normally lacking specific fluorescence, have been found not to possess this basic property (see also Norberg and Hamberger 1964), nor do the non adrenergic terminals (Malmfors 1965)

The adrenergic ganglion cells showed further a high specificity in taking up and/or decarboxylating L-DOPA In normal animals, any DOPA taken up should be rapidly decarboxylated to DA by the high DOPA decarboxylase activity which in all probability is present in these cells (Anden, Magnusson and Rosengren 1965) After the administration of DOPA to reserpine-treated animals, accumulation of fluorescent material was found only after pretreatment with nialamide There is little doubt that the effect of this drug is due to an inhibition of MAO since a strong accumulation could be obtained after α methyl DOPA — like after α methyl NA — even without such pretreatment The α methylated amines are not readily attacked by MAO and can thus accumulate intraneuronally to high levels (*cf* Carlsson 1964a Malmfors 1965) Even after pretreatment with nialamide, however, accumulation following the administration of L-DOPA could be largely prevented by administration of the potent DOPA decarboxylase inhibitor NSD 1015 (*cf* Carlsson 1964a) Since NSD 1015 does not inhibit the uptake of DA by adrenergic nerves (Malmfors 1965) and evisceration did not prevent accumulation there is little doubt that it is mainly DOPA itself and

not DA that is taken up into the neuron It thus appears that the adrenergic neurons have a fairly efficient mechanism for the uptake of L-DOPA but no observable capacity to accumulate this amino acid even after quite large doses

The uptake resulted always in a fluorescence of fairly even intensity, without the variation normal among the cell bodies All the cell bodies of adrenergic neurons thus seem to take up DOPA or CA to about the same extent, and the amines are probably localized to a much higher degree than normal in the extragranular cytoplasm, the storage function of the granules having been blocked by reserpine They seem to be capable to penetrate even into the nucleus There is also little doubt that the exogenous CA taken up are readily accessible to degradation by MAO (see above) These results and the finding that chemically quite different MAO inhibitors induced an increased CA fluorescence in the adrenergic neuron all are in agreement with other histochemical evidence for the presence of MAO intraneuronally or possibly just outside the surface membrane (Fuxe and Hillarp 1964, Hamberger *et al* 1964, Hamberger and Masuoka 1965, Malmfors 1965) There are other data also, which strongly indicate an intraneuronal localization of MAO (see Carlsson 1964a, Kopin 1964)

Serotonin (5-HT) has been shown to modulate transmission in the sympathetic ganglia of the rat (Trendelenburg 1957), but this substance has not been found in normal ganglia either by bioassay (Gaddum and Giarman 1956) or by the present histochemical method (see Norberg and Hamberger 1964) There is no doubt that adrenergic nerves are able to take up 5-HTP and 5 HT and to accumulate and store 5-HT (Andén 1964b Owman 1964) The present results give no clear evidence for such an uptake, but it is possible that a small accumulation would not be detectable Fluorescence microspectrophotometric measurements with an apparatus according to Caspersson indicate that some of the fluorescence in the ganglion cells after the administration of 5-HTP is due to 5 HT (or 5-HTP) (Ritzén personal communication) The present experiments clearly show however that the adrenergic neuron has a marked specificity as regards the uptake and/or decarboxylation of the monoamine precursors DOPA and 5-HTP This is an unexpected finding since it often has been assumed that 5-HTP could be readily taken up and decarboxylated to 5-HT by CA forming cells, such as the peripheral and central adrenergic neurons This assumption is based mainly on the view that DOPA decarboxylase is identical with 5-HTP decarboxylase, or at least that the decarboxylase of monoamine-containing cells can readily use both amino acids as substrates (see Andén 1965) If this is correct the observed specificity should lie in the uptake of the amino acids It cannot be excluded however that 5-HTP is a poor substrate for the DOPA decarboxylase of the adrenergic neurons *in vivo*

The same specificity as the peripheral adrenergic neuron shows also the central CA and 5-HT neurons (Fuxe 1965 and the CA forming mast cells of the hamster (Adams-Ray Dahlström and Sachs 1965 unpublished observations It thus seems that a specificity as regards the uptake (or possibly decarboxylation of DOPA and 5 HTP is a general property of both the nervous and non nervous cells that form and store monoamines

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Studies on Some Systems of Adrenergic Synaptic Terminals in the Abdominal Ganglia of the Cat

By

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Abstract

mission

It is generally accepted that nerve impulses from the preganglionic nerves in autonomic ganglia are transmitted by acetylcholine (for references, see McLennan 1963). An adrenergic influence on ganglionic transmission has also been suggested by several investigators. Thus injected or perfused catecholamines (CA) have been found to inhibit or enhance transmission (for references, see Goffart 1957, Curtis 1963), but such studies have failed to demonstrate that CA are released in the ganglia or play any role physiologically. Endogenous release of CA from chromaffin cells has also been proposed to influence ganglionic transmission (Eccles and Libet 1961).

Studies with the histochemical method of Falck and Hillarp revealed the presence of typical adrenergic nerve terminals in the prevertebral sympathetic ganglia of the cat (Hamberger, Norberg and Sjöqvist 1963). These terminals were found to surround the sympathetic ganglion cells in basket-like synaptic structures. Similar nerve terminals —in close contact with cell bodies or processes—have also been demonstrated in other sympathetic ganglia (Hamberger, Norberg and Ungerstedt 1965) and in the parasympathetic ganglia of the intestine (Norberg 1964) and the intramural ganglia in

studied) Synaptic fluorescent terminals could still be observed in the inferior mesenteric ganglia (8 of 8 cats studied), while very few (1 of 5 cats) or no (4 of 5 cats) terminals were found around the intramural ganglion cells in the corresponding part of the colon No or very few adrenergic vasomotor terminals were found in the denervated part of the colon The intramural ganglia of the bladder were of normal appearance

2 *Bilateral resection of the paravertebral ganglion chains L3 to L7 (S1) (3 cats) + celiac ganglia (2 cats)* Large numbers of fluorescent terminals could be observed in the inferior mesenteric ganglia, both when only the paravertebral ganglia were resected and when in addition the celiac ganglia were removed After resection of the ganglionic chains the intramural ganglia of the small intestine and of the colon still exhibited an about normal number and distribution of fluorescent synaptic terminals When the celiac ganglia had also been removed, no (1 of 2 cats) or very few (1 of 2 cats) fluorescent synaptic terminals were found in the intramural ganglia in a part of the small intestine corresponding to the nervous outflow from the resected ganglia About normal numbers of fluorescent terminals with a normal distribution were found in the intramural ganglia and muscle layers of the bladder trigonum

3 *Bilateral section of the hypogastric nerves (6 cats)* The central end of the cut nerves showed a very strong fluorescence, indicating a massive accumulation of NA (Fig 2b) No (5 of 6 cats) or very few (1 of 6 cats) fluorescent fibres were found in the peripheral end (Fig 2d) Nerve cells were sometimes observed with the green or yellow green fluorescence typical of the cell bodies of adrenergic neurons (Norberg and Hamberger 1964) Synaptic fluorescent terminals of approximately the same number and distribution as in the normal animals were found in the inferior mesenteric ganglion In the trigonum of the bladder, a striking decrease was observed in the number of visible adrenergic terminals in the muscle layers as compared with control animals, while the synaptic terminals in the intramural ganglia were of about normal distribution and number No appreciable decrease in the number or distribution of visible adrenergic vasomotor terminals was found in the bladder after this operation No decrease was observed in the number of fluorescent terminals in the intramural ganglia of the intestine

4 *Bilateral section of hypogastric and pelvic nerves (3 cats)* The central cut ends of both the hypogastric and a certain number of fibres in the pelvic nerves showed a very intense fluorescence, indicating accumulation of NA Synaptic fluorescent terminals of approximately normal number and distribution were observed in the intramural ganglia of the bladder The adrenergic innervation of the muscle layers in the trigone region had about the same low density as after sectioning of the hypogastric nerves alone No or very few adrenergic vasomotor nerves could be observed in the bladder wall after this operation, in contrast to findings after the hypogastric nerves only had been sectioned

Discussion

The specificity of the fluorescence reaction under the conditions used has been well established, and it permits direct histochemical studies on the sympathetic adrenergic neuron (see *e g* Norberg and Hamberger 1964) The experimental approach used in

the present investigation is based on two findings, which together provide a safe basis for determining the course of adrenergic nerves

1 Adrenergic non terminal axons, which are normally difficult to identify because of their low NA content and consequently low fluorescence intensity, can be easily demonstrated after axotomy (Dahlstrom and Fuxe 1964) A primary CA, recently shown to be the adrenergic transmitter NA (Dahlstrom and Haggendal 1965, unpublished data), accumulates proximally to the lesion, giving an extremely intense fluorescence, while no or only a very small such accumulation is found peripherally (Dahlstrom 1965)

2 After axotomy, the adrenergic transmitter disappears in the nictitating membrane of the cat within 36 to 48 hrs (Kirpekar, Cervoni and Furchgott 1962) Although the adrenergic vasoconstrictor nerves in cat skeletal muscle require 4 days to reach low NA levels (Sedvall 1964), the present results have not shown any difference between the disappearance of NA from nerves in denervated organs after 3 days and after longer degeneration times The risk of axon regeneration is minimal in that survival times can be kept comparatively short

The present denervation experiments, and the fact that no cell bodies of adrenergic neurons (see Norberg and Hamberger 1964) have been found in the intestinal wall (Norberg 1964) permit the conclusion that the adrenergic synaptic terminals in the *intramural ganglia of the colon* have their trophic centre on the central side of the colonic nerves The experiments also strongly indicate that their trophic centres are not located in the celiac ganglia or in the sympathetic chain ganglia, or in the spinal cord with axons traversing the ganglion chain Thus, there seems to be little doubt that the trophic centres of the adrenergic synaptic terminals in the colon — like those of the vasomotor terminals — are located in the inferior mesenteric ganglia, in agreement with the classical view that the postganglionic sympathetic nerves to the colon come from these ganglia (Langley and Anderson 1895—1896, 1896) An important part of the sympa

finally the intramural postganglionic parasympathetic neuron in the intestinal wall In this way the sympathetic and parasympathetic divisions of the autonomic nervous system are intimately interconnected in a previously (*cf* Kuntz 1953) unknown manner The adrenergic innervation of the small intestine may involve a corresponding neuron chain via the celiac ganglia The sympathetic inhibition of intestinal motility has previously been generally thought to stem from a direct effect on the smooth muscle cells (Hill 1927), but it has also been proposed that sympathomimetic amines act by blocking or depressing transmission in the intramural ganglia (McDougal and West 1954 Kosterlitz and Robinson 1957) The present results give strong support for the view that such an effect can be the main mechanism for adrenergic inhibition of intestinal motility

The trophic centre of the adrenergic synaptic terminals in the *intramural ganglia of the bladder* cannot be mainly located in the inferior mesenteric ganglia as judged from the present denervation experiments, nor can the fibres reach the bladder via the pelvic nerves — at least not to any major extent The terminals belong in all probability to the peripherally located adrenergic neurons in the bladder ganglia, or possibly to adrenergic neurons in the pelvic ganglia (*cf* Norberg and Hamberger 1964 Fig 28—29) Another possibility would be the peripheral parts of the hypogastric nerves where

nerve cells — now shown histochemically to belong to adrenergic neurons (Fig 2d)— have been reported to occur in various species (Langley and Anderson 1895—1896 Trumble 1933—1934, *Vanov and Vogt 1963)*

The adrenergic terminals innervating *the muscle layers in the trigonum of the bladder*, on the other hand, seem to have their main origin in the inferior mesenteric ganglia It *cannot be excluded, however, that a part* of the nerves to the trigone muscle belong to nerve cells in pelvic or intramural ganglia or in the hypogastric nerves The adrenergic vasomotor fibres reach *the bladder at least* to a large extent via the pelvic nerves That sympathetic unmyelinated fibres reach the bladder via the mainly parasympathetic pelvic nerves has previously been proposed by Kuntz and Moseley (1936)

The adrenergic terminals of the bladder are thus partly of intrinsic and partly of extrinsic origin, the latter reaching the bladder via the hypogastric and pelvic nerves *The extremely* complicated organization of the bladder innervation evidently explains why *such* highly conflicting results have been obtained in physiological investigations *on the bladder* (for references, see Ingersoll, Jones and Hegre 1954) It remains to reevaluate the existing physiological and pharmacological (*e g* Sigg and Sigg 1964) *data in the light* of findings with the present method

The adrenergic synaptic terminals in the *inferior mesenteric ganglia* do not have their trophic centres in the celiac ganglia or in the sympathetic ganglionic chains as judged from the present denervation experiments The trophic centres of these terminals are probably not located on the peripheral side in the effector organs since the terminals were not diminished in the ganglia after section of the hypogastric or colonic nerves *no accumulation of NA was found in any of the fibres of the peripheral* stump of the hypogastric nerve and no nerve cells of adrenergic neurons have been found in the colon (see above) and no accumulation of NA was found in any of the fibres of the peripheral *stump of the colonic nerve after axotomy The possibility that these terminals originate* from several of the organs connected with the ganglia was contradicted by the lack of NA accumulation in the peripheral cut ends of the hypogastric and colonic nerves (The *effect of a total denervation of the inferior mesenteric ganglion could not be studied* since this operation proved to cause a disturbing increase in cellular and axonal fluorescence which prevented detection of the remaining terminals) However the experiments strongly indicate that the adrenergic synaptic terminals in the *inferior mesenteric* ganglia originate at least to a major extent in the ganglia proper or possibly from ganglion cells in the hypogastric nerves (see above) It has been proposed (Kuntz and Saccamanno 1944, Mc Lennan and Pascoe 1954) that afferent nerves from the intestine to the inferior mesenteric ganglion mediate an inhibitory intestino-intestinal reflex The present results strongly indicate that such nerves are not adrenergic in nature Prominent dendritic systems have been reported in sympathetic ganglia Cajal 1906 Elfvin 1963a b) and dendritic nests have been observed around adjacent ganglion cells in the prevertebral ganglia (Kuntz 1938 1940) Some of these structures may be identical with the adrenergic synaptic terminals in the inferior mesenteric and celiac ganglia

The morphological observations are insufficient to allow any definite conclusions as to function of the adrenergic ganglionic synapses There are some indications for the existence of adrenergic mechanisms in certain parasympathetic ganglia (McDougal and West 1954, Kosterlitz and Robinson 1957 and in sympathetic ganglia Marrazzi 1939 Lundberg 1952, Costa *et al* 1961 Weir and McLennan 1963 These may have a general inhibitory function, although some contrary results have been obtained for the

latter ganglia (Bulbring and Burn 1942, Bulbring 1944). More direct evidence is needed to elucidate the function of the adrenergic ganglionic synaptic terminals, which appear to constitute widespread systems of great physiological and pharmacological importance

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The Effect of Ethyl Alcohol on Non-Gustatory Receptors of the Tongue of the Cat

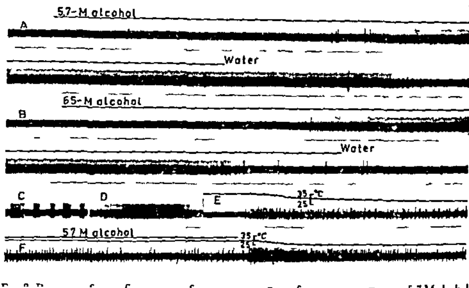
By

GÖRAN HELLEKANT

Received 9 February 1965

Abstract

The rather small response of the chorda tympani nerve to the application of alcohol on the tongue as reported in previous papers (Hellekant 1965 a, b) in comparison with the strong response to the conventional taste solutions, raises the question of an alcohol effect on sensory receptors other than the gustatory cells within the taste buds. Further the application for example of 4.9 M alcohol on the tip of a lightly anesthetized cat's tongue produced rather strong muscular reflexes which might be elicited from sensory fibres belonging to the trigeminal part of the lingual nerve, as similar reflexes were never observed during the exposure to conventional solutions. The present work was undertaken to study this response to alcohol in the trigeminal fibres of the lingual nerve.



lowest alcohol concentration during the flow of which the discharge ceased It can be concluded from the table that the alcohol concentration which caused the fibre to dis charge maximally usually caused a cessation of the discharge during the prolonged flow Column D gives *the interval between the onset of the alcohol flow over the tongue* and the moment when the induced discharge stopped although alcohol was still flow ing Column E gives the conduction velocity which ranged from 5 7 to 18 0 m sec

Fig 2 is a recording from a typical fibre in this group It is fibre no 15 in Table I The recording demonstrates some of the features which are not given in the table These fibres were characterized by the regular discharge in their response to alcohol

was usually sudden The block was reversible and the ability to discharge to alcohol returned after a while when the tongue was rinsed with water The recording shows also that the discharge ceased after about 20 sec when the tongue was rinsed with 6 5 M alcohol but not when it was rinsed with 5 7 M alcohol The recording may give a *wrong impression for it seems that the duration of the flow of* 5 7 M alcohol was shorter than that of 6 5 M but 6 sec of the recording during the 5 7 M alcohol flow is excluded in the figure In all the tongue was exposed to alcohol for a longer time in Fig 2 A than in B Fig 2 C and D shows the response to mechanical stimulation The receptor responded to light touch on a very limited (about 2 mm²) area about 5 mm from the tip of the tongue Fig 2 C shows the response to repeated touch and D to increasing and decreasing pressure

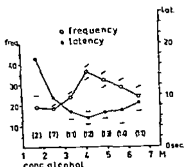


Fig 3 The circles represent the mean value of the frequency elicited by the alcohol concentrations marked on the abscissa The number of fibres is put in parenthesis below The oblique lines represent the S E The dots indicate the mean value in sec of the intervals between the onset of alcohol flow and the observed response The horizontal lines represent the S E The same number of fibres is included as for the frequency

The response to cooling from 35° to 27° C was recorded in Fig 2 E This temperature decrease caused an initial discharge in the fibre of about 10 imp sec Fig 2 F is a recording from the same fibre after 35 sec flow of 5 7 M alcohol over the cat's tongue The discharge during the last sec before the water flow was about 6 imp/sec The temperature decrease in F from 30° to 25° C immediately following the alcohol flow elicited a discharge of at least 30 imp/sec, which must be regarded as much stronger response than in E though *the temperature decrease was smaller A similar increase of sensitivity* was observed to mechanical stimulation after alcohol exposure

The summated recording in Fig 1 revealed a decreasing response to increasing alcohol concentrations stronger than 4 9 M The same observation was obtained in single fibres as Fig 3 shows The frequency was plotted on the left ordinate in the figure and the molar concentration on the abscissa The circles represent the frequency during maximum steady response as previously defined Each symbol represents the mean value *of the number of units indicated by the figures put in parenthesis below The* oblique lines show the S E. The diagram supports the conclusions made from Fig 1 that there is a decrease in the response in these fibres to higher alcohol concentrations Fig 3 shows also the intervals between the onset of a flow of alcohol over the tongue and the observed response in the fibres This latency was measured in sec and plotted on the right ordinate in Fig 3 The dots symbolize the mean value of the latency for the number of fibres indicated by the figures put in parenthesis The same number of fibres was included as for the frequency The horizontal lines indicate the S E. The shortest latency was observed for that alcohol concentration which elicited the strongest response

It was always observed during the study that fibres which responded to mechanical stimulation and not to cooling were not stimulated by alcohol Fig 4 is a recording from such a specific fibre The fibre was only sensitive to mechanical stimulation as

response The recording in Fig 4 C was obtained after 15 sec flow of alcohol This part of the recording during alcohol stimulation was chosen as the probability of a response was greatest after this time *The alcohol in this recording did however, flow over the*

receptors are situated at an average depth of about 0 18 mm in the tongue of the cat A similar more or less superficial level of other sensory nerve endings is very likely

This study supports the view suggested in an earlier study (Hellekant 1965) that alcohol penetrates the epithelium of the tongue The increased sensitivity and the reversible paralysis observed, can not be explained as an osmotic effect only, but as an interference with the receptor, which may cause a change of the permeability, similar to that observed by Knutsson (1961) in the frog muscle

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From the Institute for Medical Biochemistry, University of Oslo, Norway

The Action of Adrenaline in Cardiac Muscle

Dissociation between Phosphorylase Activation and Inotropic Response

By

Ivar Oye

Received 12 February 1965

Abstract

Oye, I *The action of adrenaline in cardiac muscle Dissociation between phosphorylase activation and* [illegible]

[illegible] phorylase activation from the time of onset of the inotropic response, phosphorylase activation being the later of the two changes and therefore unlikely to play a part in the chain of events leading to increased force of contraction In the early phase of the inotropic response no activation of phosphorylase was found when the tissue was extracted in glycerol at 30° C. [illegible]

Adrenaline stimulates the utilization of carbohydrates, lipids and oxygen by cardiac muscle The expected result of these metabolic effects would be an increase in energy available for the contractile process The idea that this increase in energy liberation is the cause of the increased contractile force was rejected by Ellis (1959) who demonstrated an inotropic response to adrenaline in isolated frog hearts under anaerobic conditions and after poisoning with iodoacetate As an alternative hypothesis he proposed that increased levels of hexose phosphates following the increase in phosphorylase activity might trigger the inotropic response Subsequent studies have lent some support to this hypothesis (Belford and Feinleib 1962, Williamson 1964

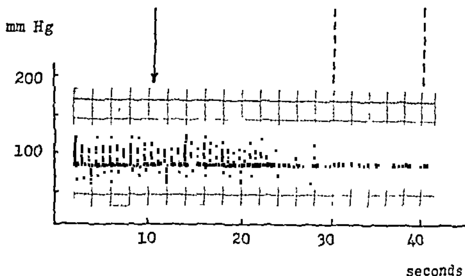


TABLE I Effect of adrenaline on phosphorylase activity All hearts were perfused for 30 min before the addition of 1 μg adrenaline to the perfusate No additions were made to the controls Phosphorylase activity assayed without added AMP (basal activity) and with AMP (total activity) The relative amount of phosphorylase a is expressed as basal activity in per cent of total activity The number of experiments are given in paranthesis Phosphorylase activity is expressed as μmoles inorganic phosphate (P_i) liberated per min g tissue (wet weight)

	Phosphorylase activity		Basal activity in per cent of total
	Basal	Total	
	μmoles P_i/min/g[1]		
Controls (8)	0 9±0 1	18 0±0 5	5 0
Adrenaline, frozen during the first 20 seconds of inotropic response (8)	0 9±0 1	17 4±0 8	5 2
Adrenaline, frozen at maximal inotropic response (8)	3 6±0 7	18 8±2 0	19 2

1 Mean value ± standard error of the mean

traction seen when adrenaline is perfused through the heart, must therefore be due to

phosphorylase was found (Table I) On the addi

TABLE II The effect of two different extraction procedures on the amount of phosphorylase a (basic activity in per cent of total activity) in control hearts and hearts frozen during the first 20 sec of inotropic adrenaline response Six hearts from each group were stored at — 80 C as frozen powder Two samples from each heart were weighed out and one extracted in 30 per cent glycerol at — 20°C, the other by the aqueous extraction procedure described in the text Phosphorylase activity expressed as in table I

Extraction procedure	Basal activity in per cent of total[1] Controls	Adrenaline	Difference	P
Glycerol extraction (6)	7 0±0 8	6 5±0 7	−0 5	—
Water extraction (6)	24 9±3 1	31 1±3 4	6 2	0 01

[1] Mean value ± standard error of the mean

TABLE III The effect on the amount of phosphorylase a found in heart tissue of adding adrenaline or cyclic 3 5 AMP before extraction of tissue Frozen tissue (control hearts) thawed by grinding in a mortar 1 min in water at room temperature a) no addition, b) adrenaline added (0 1 µg per ml), c) cyclic 3 5 AMP added (10 ⁻⁴ M) Following this treatment, the standard extraction medium was added (Final volume 10 ml per 50 mg tissue) Amount of phosphorylase a expressed as basal activity in per cent of total as described in the legend for table I

Additions during aqueous extraction	Basal activity in per cent of total[1]
a) none (4)	24 0±2 1
b) Adrenaline (0 1 µg per ml) (4)	23 2±2 4
c) Cyclic 3 5 AMP (10 ⁻⁴ M) (4)	24 1±2 2

[1] Mean value ± standard error of the mean

tion of 1 µg adrenaline at this time, a marked increase in the amount of the AMP-independent form (phosphorylase a) was always seen, provided the hearts had been frozen at the maximum of inotropic response

The chronological relationship between phosphorylase activation and inotropic response was studied more closely by freezing a series of hearts between 10 and 20 sec after the first manifestation of inotropic effect (Fig 2, the time interval marked by the two vertical broken lines) During this initial phase of inotropic response, the levels of phosphorylase did not differ significantly from the levels in the control group (Table I)

Table II reveals the importance of the extraction procedure In order to study the effect of thawing the frozen tissue in the absence of ethylenediaminetetraacetate and sodium fluoride, the extraction procedure was modified as follows Distilled water was

used instead of cold glycerol, and 50 mg frozen tissue was extracted by grinding at room temperature in 2 ml water for 1 min before addition of 8 ml of the standard extraction fluid Thawing the tissue particles to room temperature for one minute in this manner increased the total (apparent) amount of phosphorylase a In addition the observation was made that the use of this procedure resulted in a significant difference between control hearts and hearts having been exposed to adrenaline for less than 20 sec subsequent to the manifestation of the inotropic response This increase in phosphorylase a after extraction at room temperature was not due to the presence of adrenaline during the extraction procedure, nor to the presence of increased amounts of cyclic 3 5 AMP (Table III)

Discussion

In studies relating the inotropic effect of adrenaline to its metabolic effects it is usually assumed that the inotropic effect is caused by a direct action of adrenaline on the myocard However, changes in rate or rhythm may also lead to changes in force of contraction (Blinks and Koch Weser 1961) An increase in rate may cause an apparent increase in force (positive inotropic effect of activation) or a decrease in force (negative inotropic effect of activation) depending on the conditions used Thus the inotropic effect of adrenaline may actually be secondary to its chronotropic action which is related to its local action on the pacemaker and unrelated to its action of the myocard If the pacemaker is more sensitive to adrenaline than the rest of the cardiac muscle small doses of adrenaline might be expected to change contractile force without having any direct effects on the bulk of heart muscle In this case phosphorylase activation due to a primary action of adrenaline cannot be expected

In the present experiments increased rate was produced by local application of adrenaline to the pacemaker region Under these conditions the increase in rate was not accompanied by any observable increase in force of contraction The inotropic effect obtained when adrenaline was perfused through the coronary vascular bed must therefore be due to a direct effect on the myocard

The degree of phosphorylase activation in a muscle preparation may be influenced by other factors in the experimental procedure than the direct action of adrenaline The work of Bueding on smooth muscle has shown that even gentle manipulations prior to homogenization may bring about phosphorylase activation in isolated smooth muscle treated with adrenaline (Bueding *et al* 1962) In experiments preliminary to the ones presented here we made similar observations for the heart Fixation techniques which involved manipulation of the tissue gave increased levels of phosphorylase a The difference between adrenaline-treated and control hearts was also increased Consequently the heart was mounted in such a way that direct freezing in the Wollenberger clamp without any previous manipulations could be performed The perfusion technique used also allows recording of the inotropic response without stretching the heart and without severing the myocard by attaching a strain gauge or other recording devices directly to the muscle This again reduces the possibilities of artifacts brought about by handling the tissue These precautions probably contribute to the relatively low amount of phosphorylase a found in the present experiment

The observed time lag for phosphorylase activation excluded this process and the metabolic changes brought about by it from being causally related to the inotropic effect of adrenaline in the perfused heart The same conclusion has previously been

reached by Mayer *et al* (1963) by examining the dose response relationship for these two parameters

Phosphorylase b is rapidly converted to phosphorylase a during water extraction at room temperature This conversion is an ATP-dependent reaction catalyzed by phosphorylase b kinase The latter enzyme has been extensively studied by Krebs *et al* (1964) and has been found to exist in an active as well as an inactive form Accordingly the rate of conversion of phosphorylase b to phosphorylase a during extraction most likely depends on the activity of phosphorylase kinase When the hearts had been exposed to adrenaline during perfusion the formation of phosphorylase a during extraction was enhanced even when the hearts had been frozen during the first 20 sec of the inotropic response As addition of adrenaline or cyclic 3 5 AMP during the extraction was without effect an activation of the kinase must have taken place during the early phase of inotropic response and prior to freezing of the muscle

It is thus possible to follow the chronological sequence of three separate events related to the glycogenolytic effect of adrenaline An increase in cyclic 3 5 AMP levels occurs during the first seconds of inotropic response (Oye *et al* 1964) This is followed by increased activity of the phosphorylase activating system (phosphorylase kinase) Conversion of phosphorylase b to phosphorylase a occurs relatively late and cannot be detected at all during the first 20 sec of the inotropic response

The activation of phosphorylase on thawing the frozen muscle and the enhancement of this process in samples from hearts which had been exposed to adrenaline, might offer an explanation of the discrepancies between previous works relating the inotropic effect of adrenal ne to its effect on phosphorylase

Although dissociated the effects of adrenaline on phosphorylase activation and contractile force are not necessarily mediated through separate adrenergic receptor mechanisms Concievably one single event brought about by adrenaline might trigger several secondary and possibly dissociable phenomena in the cell The initial event might be the rapid accumulation of cyclic 3 5 AMP brought about by the action of adrenaline on cell membrane adenyl cyclase Conclusive evidence for this hypothesis however is still lacking

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The Adrenergic Innervation of the Eye as Demonstrated by Fluorescence Microscopy

By

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Abstract

[illegible]

accepted innervation of the vessels (particularly in the vascular layer of the eye) and of the dilator, there were found also an adrenergic innervation of the sphincter and an adrenergic ground plexus among the capillaries in the choroid and the ciliary body There was not, on the [illegible]

[illegible]

Several important problems concerning the adrenergic innervation of the eye remain, however, to be solved It is not clear to what extent adrenergic vasomotor nerves are distributed to the various parts of the vascular bed in the retina, the optic nerve and the choroid and what importance they have for *e g* the blood flow in the eye and the intraocular pressure It is still unsettled whether or not there are any adrenergic nerves going to the ciliary body It would be of great interest to know whether there are [illegible]

against the view that the two iris muscles are controlled by a reciprocal innervation from both the sympathetic and parasympathetic system Pharmacological experiments on the isolated iris (Hess and Koella 1950, Schaeppi and Koella 1964, Takáts 1964) and physiological studies on the nervous control of the pupil (Joseph 1921)

suggest the existence of an inhibitory adrenergic innervation of the sphincter muscle This, however, has been strongly denied by Lowenstein and Loewenfeld (1962) Neurohistological investigations have given some support for such a view (for references, see Stohr 1957), but it has recently been called seriously into question also on the basis of electron microscopical observations (Richardson 1964)

The adrenergic nerves can now be studied in an entirely new way by means of the fluorescence method of Falck and Hillarp, which permits the direct demonstration of the adrenergic transmitter noradrenaline (NA) (For references see Norberg and Hamberger 1964) Thanks to their high content of transmitter, the adrenergic terminals can be easily visualized and their distribution within the tissues readily examined The method has been used in this laboratory for studies on the characteristics and basic fundamental properties of the peripheral adrenergic neuron and its terminals and to elucidate the constitution of the adrenergic innervation apparatus (Norberg and Hamberger 1964, Malmfors 1965, Malmfors and Sachs 1965a and b)

The eyes of several mammals — mainly rat, rabbit and cat, and to a lesser extent also mouse, guinea pig and monkey — have been examined in the present study and *the distribution of the adrenergic nerves in the iris ciliary body, choroid, retina* and optic nerve is described below The rest of the bulb and the extraocular structures were not included in this investigation *Some observation on the adrenergic innervation of the* dilator muscle in the rat iris (Falck 1962) and rabbit choroid (Haggendal and Malmfors 1965) have been reported previously and certain special details of the adrenergic innervation of the iris in albino rat, mouse and guinea pig are summarized also elsewhere (Malmfors 1965, Malmfors and Sachs 1965a and b) [1]

Material and methods

The eyes from adult animals of the following species were examined (numbers of animals in [illegible]

[illegible]

[2] examined 48 to 96 hrs after bilateral extirpation of the superior cervical gangl[illegible]on together wi[illegible] 5 to 6 mm of the preganglionic sympathetic trunk

[illegible]

[illegible] at 5 to 10 μ the sections then being mounted for fluorescence m[illegible]croscopy f[illegible]r details [illegible]

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[1] During the preparation of the manuscript two brief reports were publis[illegible]ed on the a[illegible]renerg[illegible]c innervation of the eye studied by the fluorescence method of Falck and Hillarp ([illegible]hinger 19[illegible], Laties and Jacobowitz 1964

The technique used has previously been decribed in detail (Malmfors 1965) Fluorescence

after staining consecutive sections or whole mounts according to van Gieson's staining method (hematoxyline oxyfuchin and picric acid) Numerous sections and whole mounts were examined and photographed first in the fluorescence microscope and then in a Zeiss photomicroscope with phase-contrast equipment

Results

The chemistry and specificity of the fluorescence reaction are now well known (for references see Norberg and Hamberger 1964) The fundamental characteristics of the adrenergic neuron the intraneuronal distribution of the transmitter and the identification of the adrenergic nerve terminals have recently been discussed in other papers from this laboratory (Norberg and Hamberger 1964, Malmfors and Sachs 1965a) The results obtained show clearly that in normal mammalian tissues the adrenergic nerves — in contrast to the cholinergic and other non adrenergic nerves — develop a specific green (to yellow green) fluorescence during the histochemical treatment and that this is due to the presence of NA, which is converted to an intensely fluorescent 3 4-dihydroisoquinoline

The adrenergic nerve terminals in the eye tissues from all the species studied presented the typical appearance, which has earlier been described from other organs (Norberg and Hamberger 1964) They are fine fibres with abundant varicosities, which exhibit a strong fluorescence In contrast to this the adrenergic non terminal axons (*cf* Malmfors and Sachs 1965a) which run in the tissues in small nerve bundles are thicker and usually smooth, developing at the most a weak fluorescence In some species at least their transmitter content can be increased by treating the animals with a potent monoamine oxidase inhibitor (*e g* nialamide) This circumstance was utilized in the present study to make them easier to observe

is thus no doubt that the nerves observed in the eye tissues belong to the sympathetic adrenergic system The denervation experiments performed show also that all — or practically all — of the adrenergic fibres in the eyes of the species examined originate from ipsilateral cervical sympathetic ganglion cells and that usually very few such cells are localized peripherally of the superior cervical ganglion This has been confirmed by the examination of whole iris mounts in a much larger material from sympathectomized rats (Malmfors and Sachs 1965a) In spite of the fact that the terminals orginating from even a single nerve cell can easily be found in such preparations, almost all the irises were completely denervated following extirpation of the superior cervical ganglion

The distribution of the adrenergic nerves in the eye tissue is given below with special reference to the vascular layer Only the general and essential features are described Unless otherwise stated no obvious or important differences were found between the species examined

Outside and within the bulb sometimes in the close vicinity of blood vessels there were to be found weakly fluorescent and smooth adrenergic axons usually mixed with

non fluorescent, presumably mainly cholinergic fibres (Fig 1) These non-terminal axons gave rise to abundant terminals, distributed partly to the blood vessels The vascular innervation, which consisted of a typical ground-plexus (Fig 2) — a fine, widely meshed two-dimensional network — located on the border between the adventitia and the smooth muscle layer in the media, has been described in detail with regard to several other organs by Norberg and Hamberger (1964)

The choroid, optic nerve and retina

In the choroid, there was found — in addition to the vascular adrenergic innervation — a type of adrenergic terminals which did not seem to be directly related to the vessels These terminals — studied most easily in whole mounts of rat choroid — seemed to form a well-developed two-dimensional network (Fig 3), which to judge from the sections (Fig 4) appeared to be closely related to the capillary layer They were absent in a very narrow zone in the most anterior part of the choroid

In the optic nerve and retina, only the central retinal artery showed an adrenergic innervation, in the nerve and in the optic papilla (Fig 5, 6,) The examination of hundreds of sections revealed no fluorescent nerve fibres around either the superficial vessels or the deeper vessels of the retina (Fig 7) Apart from these vasomotor nerves no fluorescent fibres were found over and above the fluorescent, dopamine containing neurons in the retina, with intraretinal terminals (Fig 5, 7) already described (Haggendal and Malmfors 1965)

The ciliary body

In addition to an adrenergic vascular innervation of ordinary appearance, fluorescent fibres were found in the vascular layer of the ciliary body at the basis of and in the ciliary processes These formed a very finely meshed network among the blood vessels, mainly the capillaries (Fig 8) It is difficult to decide as yet whether all these nerves belong to the blood vessels or whether they also constitute a direct innervation of the ciliary epithelium In whole mounts of rat ciliary body, the adrenergic ground plexus seemed to outline the ciliary processes (Fig 9) rather than separate blood vessels and showed about the same appearance as that of the nerves in whole mounts of rat choroid which were not directly related to the vessels In the rest of the ciliary body the adrenergic nerves were sparse Some single fluorescent nerve fibres were in fact to be found in the ciliary muscle, with some variations between different species, but they seemed to be too few to constitute a real adrenergic innervation of the ciliary muscle

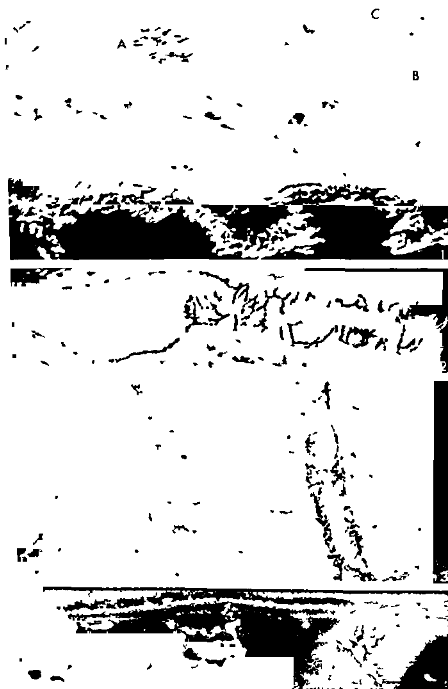
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Fig 1 Cat iris perpendicular section At the bottom is seen the dense ground plexus of the dilator In addition to the auto-fluorescent cells small nerve trunks are seen in the stroma A — fluorescent axons B — non fluorescent axons C — both fluorescent and non fluorescent axons. × 250

Fig 2 Cat iris The adrenergic ground plexus is seen around a tangentially sectioned blood vessel × 160

Fig 3 Rat choroid whole mount In addition to the adrenergic ground plexus around the blood vessels, there is a meshwork of fluorescent fibres not directly related to any large vessels × 160

Fig 4 Rabbit choroid perpendicular section Fluorescent fibres are seen among blood vessels in the capillary layer of the choroid The auto-fluorescent pigment epithelium (•) × 250



A ta physiol scand Torbjörn Malmfors

ON



The iris

In the iris, both the non-terminal axons and the adrenergically innervated vessels could be easily studied, especially in the whole mounts. In the rat, only arterioles seemed to be innervated.

In the whole mounts of iris, the adrenergic innervation of the dilator muscle was found to be composed of a ground plexus uniformly distributed over the entire muscle (Fig. 14). In each strand of the network there could be seen one or several adrenergic terminals. The terminals were of typical appearance, with very strongly fluorescent varicosities and more weakly fluorescent segments in between. For a more detailed description of the adrenergic ground plexus as revealed from whole mounts of rat iris, see Malmfors (1965) and Malmfors and Sachs (1965a and b). Certain differences were found between the species examined. In the mouse, each strand usually contained no more than one terminal, while in rat and guinea pig the strands were usually composed of several terminals. In the mouse the varicosities seemed to be slightly larger than in rat, 1.5 μ in diameter as compared to 1 μ in the latter.

In the sections of iris, the adrenergic nerves in mouse and rat were found superimposed on the anterior surface of the dilator muscle (Fig. 11, 12), which consists in these species of one cell layer only (Nilsson 1964). In these animals, no terminals were found to give off branches extending in between the muscle cells of the dilator. In the cat and rabbit, the adrenergic ground plexus of the dilator muscle was very dense and most abundant on the anterior surface of the muscle (Fig. 1, 10). But the fluorescent fibres extended also in between the muscle cells, which seemed to form more than one layer. The innervation was richer in the cat than in rabbit.

At the pupil edge of the whole mounts of rat iris, the fluorescent fibres formed a ground plexus of an appearance somewhat different from the plexus in the rest of the iris, which has earlier been described as constituting the adrenergic innervation apparatus of the dilator muscle. There was a distinct transition between these two ground plexus, but they were very intimately joined together (Fig. 14). The plexus at the pupil edge, which was situated just over the sphincter zone of the iris, was considered to form the adrenergic innervation of the sphincter muscle (see Discussion). This was more evident when the sphincter of rat was studied in transverse sections. The fluorescent fibres were then found to be sparsely but uniformly distributed between the muscle cells and somewhat more abundant on the anterior surface of the muscle (Fig. 12). No continuation of the accumulation of the fluorescent fibres above the dilator was found behind the sphincter muscle. Nor were any evident signs of dilator muscle cells found in or behind the sphincter muscle (Fig. 13).

Fig. 5. Section of the posterior half of rat eyeball, through the entry of the optic nerve (ON). Fluorescent fibres are seen around the central artery of the retina in the nerve but not further. A fluorescent nerve cell body — probably containing dopamine — in the retina is also seen. (→) × 100.

Fig. 6. Rat optic papilla. Section through the branching central artery of the retina. Fluorescent fibres are seen around the vessel in the optic nerve but no further. × 250.

Fig. 7. Monkey retina. No fluorescent fibres are seen around the blood vessel, but at the border between the inner plexiform and the inner nuclear layer are numerous fluorescent terminals from intraretinal neurons (→). × 250.

Fig. 8. Rabbit ciliary body. Fluorescent fibres are seen both in the ciliary processes and at their bases, intermingled with small blood vessels just below the epithelium. × 160.

In the cat, where it is easy to distinguish between the spincter and dilator m[illegible] the adrener[illegible] same der[illegible] 1964) or [illegible] of cat (Malmfors [illegible] [illegible] and the dilator did not seem to reach the pupil edge In the sphincter muscle, however, a fairly large number of fluorescent fibres — more in the pigmented animals (Fig 17) than in the albinos (Fig 18) but fewer than in the cat — were found uniformly distributed and intermingled among the muscle cells Fluorescent fibres which seemed to lack any connection with the dilator muscle were found also in the sphincters of monkey, mouse and guinea pig

Discussion

Since the specificity of the fluorescence method used has been satisfactorily proved by several authors (for references, see Norberg and Hamberger 1964) there is no doubt that the specific fluorescence of the nerve fibres is derived from the endogenous NA in the adrenergic postganglionic sympathetic nerves especially since the fluorescence disappeared after reserpine pretreatment and cervical sympathectomy

This paper thus specifically demonstrates the adrenergic innervation of the eye, which is of fundamental importance for the interpretation of the results of physiological and pharmacological experiments in this field It may be just the lack of such morphological data which has led to the contradictory conclusions drawn from these studies leaving the basic problems unsettled

In the present paper the main principles of the adrenergic innervation will be discussed in relation to earlier fundamental facts

The adrenergic innervation of the vessels in the eye is assumed be responsible for vasoconstrictions — e g that observed by Niesel (1961) in the choroid following stimulation of the cervical sympathetic nerve It seems unbelievable on the other hand that the retinal vessels should be directly influenced by adrenergic activity, since no evidence of an adrenergic innervation of these vessels was found in the species examined Yet

---

Fig 9 Rat ciliary body whole mount Two small nerve trunks (→) and an arteriole (A) are seen passing the ciliary body to the iris (I) Fluorescent fibres are accumulated in the ciliary processes (CP) which they seem to outline in the same way as the arteriole × 160

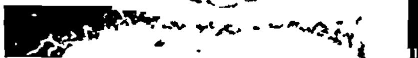
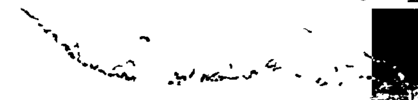
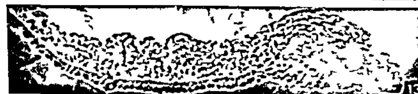
Fig 10 Rabbit iris perpendicular section The adrenergic nerves of the dilator are seen in the muscle above the iris epithelium × 250

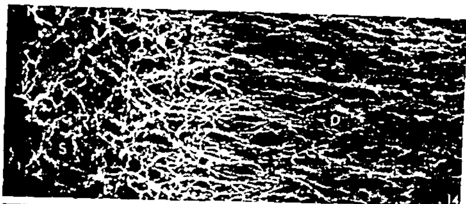
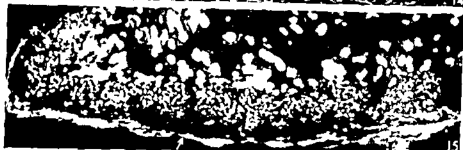
Fig 11 Rat iris perpendicular section The adrenergic nerves to the dilator are seen superimposed on the muscle Fluorescent fibres are also seen around a vessel × 250

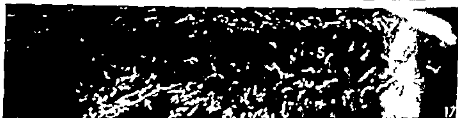
Fig 12 Rat iris radial section To the right the sphincter is seen at the pupil edge containing fluorescent fibres both among the muscle cells and on the anterior surface The accumulation of fluorescent fibres superimposed on the dilator does not continue beyond the sphincter × 160

Fig 13 The same section as in Fig 12 with phase contrast microscopy The muscles are easily identified sphincter (S) and dilator → 160


A
9

10

11

12

13


S
D
14

15

16

S
17


there was found an adrenergic innervation of the central retinal artery. This, however, stopped on the optic papilla without any special features, after having been of ordinary appearance both outside and within the optic nerve.

It is difficult to know what function can be assigned to the adrenergic ground plexus found in the choroid, which is not directly related to the large vessels but rather to the capillaries. There is no earlier evidence for a direct innervation of the capillaries and the fluorescence method cannot furnish conclusive evidence of this. The intimate relationship between the ground plexus and the capillary layer may, however, indicate some adrenergic influence on the function of the latter.

Feeney and Hogan (1961) have suggested an innervation of the melanocytes in the choroid.

It has been concluded from physiological and pharmacological experiments that there exists an adrenergic influence on the action of the ciliary body antagonistically to the parasympathetic innervation. The site of this influence, however, has been disputed. Some authors consider the effect to be due solely to vasoconstriction (Morgan 1946), while others believe in an inhibitory adrenergic innervation of the entire ciliary muscle (Melton *et al.* 1955). The latter opinion is supported by Génis-Galvez (1957), who found morphological evidence for an adrenergic innervation of the ciliary muscle. It was impossible, however, to confirm this finding in the present work and the former opinion seems more acceptable in that the adrenergic innervation of the ciliary body is accumulated to the vascular layer. The nerves, however, are not mainly situated closely around vessels in the normal manner but have a more diffuse distribution in the richly vascularized tissue at the base of the ciliary processes, so that another function of this innervation cannot be excluded.

Vasoconstriction in the ciliary body, together with vasoconstriction in the choroid and a decrease in the production of the aqueous humor, may also perhaps be responsible for the decrease in intraocular pressure observed on sympathetic stimulation (Niesel 1961). There is evidence (Eakins 1963) that the secretory process of ocular fluid is inhibited by NA. It is impossible, however, to decide from the present results whether the same effect might not be caused by a direct adrenergic innervation of the ciliary epithelium or mediated indirectly by an influence from the adrenergic nerves on the capillaries in the ciliary processes.

It is now quite clear that the dilatation of the pupil produced by stimulation of the cervical sympathetic chain is mediated directly by an adrenergic innervation of the dilator muscle of the iris (for references, see Loewenfeld 1958). The findings of the present study further support this opinion. On the other hand it has not previously been possible

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Fig. 14. Rat iris, whole mount. To the left is seen the ground plexus over the sphincter (S) and to the right the ground plexus over the dilator (D). × 160.

Fig. 15. Cat iris, radial section. In addition to auto-fluorescent cells, the sphincter is seen in the stroma containing large numbers of fluorescent fibres. The dilator (→). × 160.

Fig. 16. Cat iris. In the slightly obliquely sectioned sphincter are seen varicose fluorescent fibres running mainly singly. In the dilator the adrenergic innervation is so rich that it is difficult to see each individual fibre. × 250.

Fig. 17. Rabbit iris, radial section from an albino animal. Fluorescent fibres are seen in the sphincter (S) and dilator (→). × 160.

Fig. 18. Rabbit iris, radial section from a pigmented animal. Fluorescent fibres are seen in the sphincter (S) and dilator (→). × 100.

to decide whether there is also an active adrenergic relaxation of the sphincter during the dilatation, even if various evidence in that direction have been presented (Joseph 1921, Hess and Koella 1950, Schaeppi and Koella 1964, Takáts 1964) From the present results, however, it is clear that there is an adrenergic innervation in the sphincter It is consequently possible that the adrenergic innervation of the sphincter is inhibitory The innervation was most convincing in the cat but was also highly probable in the other animals examined

For generous supplies of drugs I am indebted to Swedish Ciba Stockholm (reserpine) and Swedish Pfizer Stockholm (nialamide)

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From the Metabolic Division, Department of Pharmacology, University of Goteborg Sweden

Hydrolysis of Adenosinetriphosphate and Creatinephosphate on Isometric Contraction of Vascular Smooth Muscle

By

ANDRAS BEVIZ, LENNART LUNDHOLM, ELLA MOHME-LUNDHOLM AND NANDOR VAMOS

Received 16 February 1965

Abstract

Beviz, A, L Lundholm, E Mohme Lundholm and N Vamos *Hydrolysis of adenosinetriphosphate and creatinephosphate on isometric contraction of vascular smooth muscle* Acta physiol scand 1965 65 268—272 In experiments on isolated bovine mesenteric artery the adenosinetriphosphate (ATP) and creatinephosphate (CrP) contents were determined after isometric contraction by adrenaline *1 min after addition of adrenaline (1 10 ⁶), when the rise of tension was one-half maximum* the ATP content had fallen by 0 16 ± 0 07 μmole/g of tissue and the CrP content by 0 22 ± 0 07

still reduced by 0 52 ± 0 18 μmole/g of tissue Calculation of the $\frac{P\ L}{H}$ quotient from the summed hydrolysis of ATP and CrP and the rise in tension during the first minute of contraction gave a value of 9 5 This value was of similar magnitude to that found on thermoelectric determination of the heat production associated with isometric contraction of striated and smooth muscle

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In A Challenge to Biochemists' Hill (1950 pointed out that the conception of adenosinetriphosphate (ATP) as the substance which furnishes the immediate energy for muscle contraction was founded upon more or less indirect experimental evidence,

(1954) to verify the postulated hydrolysis of ATP in contracting striated muscle The negative outcome undermined for a time the belief in the primary role of ATP in muscle

contraction Davies Cain and Delluva (1959) and Cain and Davies (1962) then studied a number of other phosphate compounds to find out if any of them were hydrolyzed during contraction but their results too were negative Cain Infante and Davies (1962), however, did succeed in demonstrating hydrolysis of ATP on single contraction of rectus abdominis muscle from frog by inhibiting the enzyme creatinetransphosphorylase with fluorodinitrobenzene

We have determined in experiments on isolated vascular smooth muscle the lactic acid production associated with isometric contraction under anaerobic conditions The isometric contractile process, we found, required a considerable expenditure of energy The muscle had to consume roughly 3 times the amount of energy stored in its preformed high energy phosphated compounds (ATP + ADP + CrP) for the attainment of maximal tension (Lundholm and Mohme Lundholm 1965) The pre existing energy rich phosphate compound content of vascular smooth muscle was about one-sixth that of striated frog muscle (Lundholm and Mohme Lundholm 1962) Further, the maximal anaerobic lactic acid production on isometric contraction of vascular muscle at 37° C was approximately one seventh of that recorded by Cori (1956) in experiments on anaerobically contracting frog muscle at 0° C Certain of the reactions which resynthesize high energy phosphate compounds could therefore be expected to proceed far more slowly in vascular than in striped muscle Finally, the contraction rate for vascular muscle was about 100 times slower than that for frog striated muscle (Lundholm and Mohme Lundholm 1962a)

We considered it worth while to find out if the isometric contractile process of vascular muscle was accompanied by demonstrable hydrolysis of adenosinetriphosphate (ATP) and creatinephosphate (CrP)

Methods

special plastic frames that the tens on was evenly distributed over their sufraces The plastic

experiments at the end of 60 min, during which period adrenaline 10 ⁴ was added to the solut on at 15-min intervals

The portion of the arterial preparation which had developed tension was cut out from the frames Each arterial piece was immersed in a weighed volume of iced 6 per cent perchloric

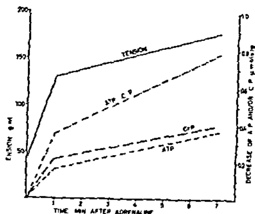


Fig 1 Rise of tension hydrolysis of ATP and CrP and summed hydrolysis of ATP + CrP in bovine mesenteric artery 1 and 7 min after addition of adrenaline at concentration of 10^{-6}

TABLE I Influence of adrenaline ($1:10^{6}$) on the content of ATP, ADP AMP and lactic acid of bovine mesenteric artery Concentration of metabolites in µmoles/g wet weight. n = number of tests p = probability that the effect was due to chance

	ATP	CrP	ATP+CrP	ADP	AMP	Lactic acid
Basal values	0 91 ± 0 10	0 66 ± 0 09	1 54 ± 0 16	–	–	–
Change after 1 min (n = 13)	−0 16 ± 0 07 $p < 0.05$	−0 22 ± 0 07 $p < 0.01$	0 35 ± 0 15 $p < 0.05$	–	–	–
Change after 7 min (n 13)	−0 37 ± 0 1 $p < 0.01$	−0 40 ± 0 09 $p < 0.001$	−0 78 ± 0 16 $p < 0.001$	0 01 ± 0 12 (n = 5)	−0 11 + 0 11 (n = 5)	1 0 ± 0 17 (n = 5) $p < 0.01$
Change after 60 min (n 5	−0 01 ± 0 12	−0 52 ± 0 18 $p < 0.05$	0 53 ± 0 28	–	–	–

The method used for determination of ATP was not specific it determined other nucleotide triphosphates as well On paper chromatographic analysis however only ATP was demonstrable in extracts from mesenteric arteries (Lundholm and Mohme Lundholm 1962 b

Results

Adrenaline produced a substantial elevation of tension which was half maximal after about 1 min and had reached its maximum after 7 min Fig 1 Table I The ATP and CrP contents fell almost parallel the decrease being statistically significant At the end of 7 min the magnitude of this fall was almost double the 1 min figure There was no change of ADP or AMP Lactic acid increased 1 µmole g In the 60-min experiments the increase of tension was still maximal at the end of that period the ATP content was not changed whereas the CrP content was still depressed

Discussion

The contractile affect of adrenaline on vascular muscle was attended by a decrease of the ATP and CrP contents — When, in another investigation, dihydroergotamine was used to inhibit the contractile effect of adrenaline, the latter raised the ATP content and relaxed the muscle (Beviz and Mohme Lundholm 1965) In further experiments the contractile effect of potassium ions also was found to be associated with a reduction of the ATP and CrP contents It seems likely, therefore, that hydrolysis of ATP and CrP accompanied the contractile process in vascular muscle

In the hope of securing additional evidence linking hydrolysis of ATP to the contractile process we computed the energy consumption in relation to the evolved tension and compared the result with available thermoelectric data on striated and smooth muscle Hill (1958) expressed the evolved tension in relation to energy econsumption by calculating the $\frac{P\ L}{H}$ quotient, where P is the force in g wt per cm^2 of transverse area, L is the muscle length in cm, and H is the heat production in g-cm per g of muscle From thermoelectric determinations on frog muscle Hill (1957) found that under strictly isometric conditions the quotient had a value of 10 2—11 0, whereas previous determinations under less ideal isometric conditions had given a figure of approximately 6 Bozler (1930), using thermoelectric methods in experiments on retractor pharynx from *Helix pomatia*, had arrived at a mean of 7 0 (range 5 9—9 1)

The tension 1 min after addition of adrenaline had in our experiments risen from an average of 43 to 133 g i e, by 89 (Fig 1) Since the transverse area of the muscle at the pertinent length was calculated at 0 075 cm^2, the increase of tension amounted to 1,190 g/cm^2 Concomitantly, the total ATP and CrP content decreased by 0 35 µmole/g Assuming that on hydrolysis of 1 mole ATP or CrP, 8,400 cal is liberated (Purton 1958) the amount released on contraction of vascular muscle was 0 35 8 4 10^{-3} cal per g Taking 4 26 10^4 g-cm as the mechanical energy equivalent of 1 cal, the $\frac{P\ L}{H}$ quotient for vascular muscle may be calculated at $\frac{1\ 1{,}190}{0\ 35\ 4\ 26\ 10^4\ 8\ 4\ 10^{-3}} = 9\ 5$ This value is very close to the ratios recorded by Hill in experiments on frog muscle and by Bozler in experiments on snail smooth muscle

The above calculations do not take into account the possible resynthesis of high-energy phosphate compounds which may occur during the period of contraction Lundholm and Mohme-Lundholm (1965) noted, a maximal increase of 6 8 µmoles/g/15 min (0 45 µmole/g/min) in the lactic acid production on contraction of vascular muscle by adrenaline, under anaerobic conditions With formation of 1 mole lactic acid from glucose an equivalent amount of ATP or CrP is concurrently synthesized If the resynthesizing processes had instantously started at maximal velocity — an assumption which is of doubtful validity — it may be estimated that a total of 0 8 µmole ATP + CrP per g was consumed during 1 min contraction This value for the energy metabolism would give a $\frac{P\ L}{H}$ ratio of 4 2 which is not too far removed from the values calculated by thermoelectric methods

These preliminary calculations accordingly indicate that the hydrolysis of ATP and CrP demonstrated in vascular muscle was of the order of magnitude which from

thermoelectric determinations on striated and smooth muscle, may be expected in association with muscle contraction

The ATP content was only temporarily depressed by adrenaline, at the end of 60 min it was unchanged on comparison with the controls The CrP content, on the contrary, was still reduced In other experiments on mesenteric artery the energy metabolism showed a considerably greater increase during the rise of tension than after the attainment of maximum tension (Lundholm and Mohme-Lundholm 1965) It seems likely, therefore, that the decrease of the ATP content was limited to that phase of the contractile process in which the energy consumption was greatest

We are indebted to Miss Inga Johansson for technical assistance Financial support was provided by the Swedish National Association against Heart and Chest Diseases and the Swedish State Medical Research Council

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From the Department of Physiology, Karolinska Institutet Stockholm, Sweden

Effects of Synthetic Angiotensin II on Catecholamine Levels and Biological Activity

By

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Received 18 February 1965

Abstract

Data have been presented from several laboratories suggesting a possible interrelationship between angiotensin II and the sympathetic nervous system Synthetic angiotensin II, administered via the carotid inflow to the vascularly isolated recipient's head in dog cross circulation experiments, produced pressor effects in the recipient's trunk which were believed to be due to stimulation of central sympathetic structures (Bickerton and Buckley 1961, Buckley *et al* 1963) Laverty (1963) reported that the administration of 1 μg or more of angiotensin into the systemic circulation of rats produced vasoconstriction in the vascularly isolated innervated hind limb which was abolished when the hind limb was denervated Laverty suggested that the vasoconstriction might

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the pressor activity of exogenous noradrenaline, they concluded that angiotensin II has an effect on the sympathetic division which results in an enhanced response to substances or procedures inducing a release of endogenous noradrenaline and that the effect was peripheral and did not appear to involve a sensitization of receptors and was unrelated to the direct vasoconstricting action of the polypeptide Feldberg and Lewis (1964) have reported that minute doses of angiotensin II administered i v to cats produced a contraction of the nictitating membrane which was eliminated by bilateral adrenalectomy They also found that the administration of extremely small quantities of angiotensin II into the coeliac artery induced a contraction of the nictitating membrane and concluded that angiotensin II was a potent releaser of adrenaline from the adrenal medulla Angiotensin II potentiated the height of the contractions of the vas deferens of the guinea pig induced by electrical stimulation of the hypogastric nerve but did not affect the responses to noradrenaline and acetylcholine (Benelli Della Bella and Gandini 1964) These investigators suggested that angiotensin II acted at the peripheral nerve endings by *promoting a greater output of noradrenaline* In concentrations of 0 01 and 0 1 μg/ml angiotensin II did not influence the release rate of noradrenaline from isolated bovine nerve granules (Euler and Lishajko personal communication)

This present investigation was undertaken to further investigate the interrelationship of angiotensin II and the sympathetic nervous system

Methods

Isolated perfused rabbit kidneys

Rabbits were anesthetized with 35 mg/kg of sodium pentobarbital via a marginal ear vein [illegible] lene cannula

[illegible]

to 16 ml min after which time one of the pair of kidneys [illegible] Rabbit kidneys were perfused for 1 hr after the [illegible] ghed [illegible] buffer [illegible] for 40 [illegible] d and

[illegible]

particulate fraction and the supernatant fraction The catecholamines were extra[illegible] either 5 or 10 % trichloracetic acid and estimated fluorimetrically utilizing the method of Euler and Lishajko (1961

Additional experiments were conducted on 8 kidneys in which the flow of the perfusate to the [illegible] and perfu- [illegible] essure was [illegible] 250 500 [illegible] tensin II

[illegible]

Angiotensin infusion on the catecholamine content of the heart and kidney

[illegible] of the Sprague-Dawley strain weighing between 270 and 350 g were utilized in [illegible] tensin [illegible] tensin II

[illegible]

TABLE I Catecholamine content ($\mu g/g \pm$ S E M) of isolated perfused rabbit kidney

		Control (perfused with Tyrode's solution)		Treated (perfused with Tyrode's solution + angiotensin)	
Fraction	N	NA	A	NA	A
Total content	5	0.102±0.009	0.007±0.0016	0.105±0.007	0.007±0.0013
Coarse	3	0.056±0.012	0.010±0.003	0.042±0.010	0.007±0.003
Supernatant	3	0.018±0.015	0.007±0.001	0.019±0.011	0.012±0.003
Particulate	3	0.022±0.003	0.008±0.002	0.036±0.007	0.005±0.003

solution was adjusted so that each animal received between 1.0 and 1.25 ml of solution in the hour period Sham operations were conducted on the 3 control animals in that the left common carotid artery was isolated and tied off the femoral vein isolated, and 1.0 ml of saline slowly infused i v After 1 hr of treatment the animals were killed by severing the jugular veins and the hearts and kidneys removed blotted dry, and weighed The individual organs of the treated and control animals for each experiments were pooled and the catecholamines determined in the manner previously described

Dog cross circulation studies

5 dog cross circulation preparations (Buckley *et al* 1963) were utilized in the studies Female [illegible]

time period The volume of urine produced was recorded and each urine specimen assayed for free adrenaline and noradrenaline according to the method of Euler and Lishajko (1961)

Results

Isolated perfused rabbit kidney

The perfusion of synthetic angiotensin II, in concentrations ranging from 0.02 μg/ml to 0.2 μg/ml at a rate between 12 and 16 ml/min for a period of 1 hr, did not alter the content of catecholamines in the tissue, and did not significantly alter the relative intracellular distribution of the amines (Table I) The administration of tyramine in doses ranging from 250 to 500 μg produced very mild increases in the perfusion pressure

TABLE II Effects of angiotensin infusion on catecholamine content (μg/g ± S E M) of rat hearts[1]

	Control (saline infusion)		Treated (angiotensin infusion, 1 0 μg/kg/min)	
Fraction	NA	A	NA	A
Total content	0 593±0 127	0 084±0 016	0 513±0 085	0 097±0 007
Coarse	0 217±0 026	0 040±0 009	0 197±0 026	0 038±0 005
Supernatant	0 145±0 017	0 031±0 010	0 134±0 016	0 034±0 004
Particulate	0 149±0 021	0 029±0 003	0 114±0 007	0 027±0 005

[1] $N = 4$ (groups of 3 pooled hearts)

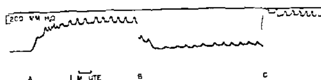
TABLE III Effects of angiotensin infusion on the total catecholamine content (μg/g ± S E M) of rat kidneys

Control (saline infusion)		Treated (angiotensin infusion 1 0 μg/kg/min)	
NA	A	NA	A
0 073±0 005	0 013±0 001	0 085±0 006	0 012±0 006

ranging from 20 to 80 mm of water The responses to tyramine during angiotensin II infusion were either equal to or slightly less than the response prior to angiotensin II infusion *The data obtained in this study indicates that angiotensin II does not have a* direct sensitizing effect on the vasculature of the kidney to the vasoconstriction induced by tyramine Likewise the response to noradrenaline was not enhanced during angiotensin II infusion and was either equal to, or much less than the response produced by equivalent doses during the perfusion with plain Tyrode's solution During this portion of the study, it was observed that angiotensin II induced rhythmic waves with a frequency ranging from 2 to 3 per minute and a pressure ranging from 30 mm of water to 120 mm of water Even when the perfusion pressure was decreased to pre angiotensin levels by decreasing the rate of flow of the perfusate these rhythmic changes in resistance were still evident but the waves produced by alterations in perfusion pressure were decreased approximately 50 % (see Fig 1)

Angiotensin II on the catecholamine content of rat heart and kidney

The infusion of 1 0 μg/min/kg of synthetic angiotensin II to 12 anesthetized rats for a period of 1 hr did not significantly alter the tissue content of catecholamines in the heart and kidney and did not significantly alter the intracellular distribution of catecholamines in the rat heart The pressor effects of this dose of angiotensin II ranged from 25 to 40 mm Hg



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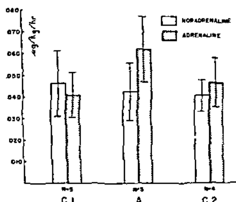


Fig 2 Urinary excretion of catecholamines in recipient dog during one hour control period (C—1), one hour during which six [illegible]

Dog cross circulation studies

The administration of the 6 equally spaced doses of angiotensin II (1 0 µg/kg of donor's weight) via the carotid inflow to the recipient's head during a 1 hr period did not significantly alter the excretion of catecholamines in the recipient's urine However, although the noradrenaline levels remained fairly constant over the 3 hr experimental period, the mean adrenaline level increased approximately 50 % during angiotensin II treatment and returned to almost control levels during the third hour control period (Fig 2)

Discussion

[illegible]

amines These data suggest that the stimulating action of angiotensin II on the adrenal medulla (Feldberg and Lewis 1964) is highly selective and that the peptide does not induce a general release of catecholamines from all organs and tissues There was no interrelationship between the activity of angiotensin II and tyramine on the vasculature of the isolated kidney which is in agreement with the conclusions of McCubbin and Page (1963) that angiotensin II does not cause sensitization of the vascular receptor of noradrenaline Spontaneous rhythmic motility of the perfused spleen has been reported to be induced by angiotensin II (Benelli, Della Bella, and Gandini 1964

In this current study, the slow alterations in vascular resistance were marked and occurred in the kidneys from 9 out of 11 young rabbits (weighing between 1 5 and 2 6 kg) These rhythmic pressure changes did not occur when the kidneys of 8 older female rabbits weighing approximately 3 5 kg were perfused with angiotensin solution in an identical manner Waugh (1964) has demonstrated that arterial pressure elevation induces rhythmic changes in blood flow due to rhythmic prevenous resistance changes Although the force of the rhythmic alterations in resistance was greater as perfusion pressure was increased, it did not appear that the increase in pressure was entirely responsible for the induced rhythm Since these rhythmic waves were still evident when perfusion pressure was manually adjusted to pre angiotensin levels it appears that the polypeptide played a role in inducing these rhythmic changes in resistance

The mechanisms by which angiotensin II produces the centrally induced hypertensive reaction (Bickerton and Buckley 1961, Buckley *et al* 1963 Laverty 1963) could not be identified in this current study However, in 4 of the 5 dog cross circulation experiments, there was a marked increase in the adrenaline excretion in the urine during angiotensin II administration whereas there was no apparent change in the noradrenaline excretion rate The possibility exists that the pressor responses observed in the recipient s trunk during the administration of angiotensin II into the vascularly isolated head may be due to the release of adrenaline The large number of variables in the cross circulation experiments which in this present study included an attempt to maintain a *fairly constant urine flow during the three hour experimental procedure, greatly* influenced the precise amount of each of the catecholamines excreted during the experimental period Therefore, although there was a relatively marked increase in the adrenaline excretion during angiotensin II treatment, the data obtained did not show a statistically *significant alteration in the excretion rate of adrenaline during angiotensin II* administration

The author wishes to thank Mrs Åge Palmfeldt—Jorgensen and Mr Nils Åke Persson for their technical assistance
This study was supported by the following research grants from the National Institutes of Health Bethesda Maryland 1 SO1 FR-05455 MH-04511 and HE-03175 and the Swedish Medical Research Council (project nr 14X 97 01

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Acta physiol. scand. 1965. 65. 279—284

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Studies on the Elimination of Exogenous Lipids from the Blood Stream

The Kinetics for the Elimination of Chylomicrons Studied by Single Intravenous Injections in Man

By

Dag Hallberg

Received 8 March 1965

Abstract

Plasma samples were drawn and analysed for TG without and with separation into 3 fractions

The elimination from the blood stream of injected chylomicrons isolated from lymph in man has been investigated by Bierman and Hamlin (1962) and Nestle *et al* (1962) The kinetics for the initial elimination was described as exponential

The kinetics for the elimination of an artificial fat emulsion from the blood stream in man has been described earlier (Hallberg 1964, 1965 a, b, c) It was the same as that found for the fat emulsion and chylomicrons in dogs (Carlson and Hallberg 1963) The elimination was governed by 2 rate constants one being an expression for a zero-order reaction at high triglyceride (TG) concentrations and the other for a first-order reaction at low TG concentrations The constant for the zero-order reaction was interpreted as an expression for a maximal elimination capacity (mmole l plasma/min) Below a

TABLE I The height and weight of 2 subjects given intravenous infusion of chylomicronpreparations The triglyceride (TG) concentration of the preparations with and without separation into 3 TG fractions in polyvinylpyrrolidone density gradient, the infused volumes and amount of TG (assumed m w 890) are set out

Case no	Height cm	Weight kg	Total TG mmole/l	TG in gradient fractions mmole/l top	middle	bottom	Infused volume ml	Infused TG g/kg b w
1	168	72	44	45 15	0 68	0 31	480	0 26
2	158	42	34	30 00	0 44	0 62	250	0 18

critical concentration" the capacity was not fully utilized and the amount eliminated per time unit was dependent on the TG concentration (cf Hallberg 1965 d)

The aim of the present study was to investigate the elimination curve of human chylomicrons from the blood stream with a density-gradient method earlier applied for a fat emulsion

The results show that the initial elimination could be linear and that no qualitative differences existed with regard to the kinetic principles found for the elimination of the used artificial fat emulsion

Material and methods

Subjects 2 subjects were chosen for the investigation Case 1 was a 62 year old woman with a stomach ulcer 3 months before the investigation she had had a rib fracture with subsequent pleural effusion The pleural fluid contained suspectedly malignant cells at microscopy The thoracic duct was cannulated for preoperative cytological diagnosis on the lymph Laboratory findings in plasma (haemoglobin, red and white cells alkaline phosphatases bilirubin GPT GOT) were normal There was no history of changes in body weight during the 3 months before the investigation The ulcer was later removed and was found to be non malignant

Case 2 was a 72 year old man with a suspected malignant ulcer in the stomach The thoracic duct was cannulated for preoperative cytological diagnosis on the lymph Laboratory findings in plasma as in case 1 were normal His body-weight had decreased by about 2 kg during 4 months before this investigation At operation later on the ulcer was found to be non malignant

The heights and weights of the subjects are set out in Table I

Procedures After an overnight fast the subjects were allowed to drink 150 ml of cream (containing 40 % fat) at 7 a m After 1 5 hrs the thoracic duct was cannulated in the left supraclavicular space with a polyethylen tube during general anesthesia induced with barbiturate Narkotal® ASTRA (5-(2 bromallyl) N-methyl 5 isopropyl malonyl-carbamide and continued with nitrous oxide and the muscle relaxing agent Celocurin® VITRUM succinyl choline) The lymph from the thoracic duct was collected in sterile 500 ml bottles with 100 ml of ACD-solution (containing citric acid 0 48 g trisodium citrate 1 32 g and glucose 1 47 g per 100 ml) to prevent coagulation The bottles were kept in ice water during the collection and heated to 25 C during 1 hr before infusion As soon as possible after the cannulation procedure the patients were urged to drink cream and take fatty meals ad lib until 8 p m The chyle was collected during 20 hrs The bottles were then centrifuged for 9000 g min at 4 C for sedimentation of blood corpuscles 40 ml from the bottom of the bottles were withdrawn for cytological examination and the rest was mixed and samples were taken for TG determination and microscopy No aggregations of fat particles were found by dark field microscopy The chylomicron suspensions were then given i v to the patients as rapid infusions about 100 ml min)

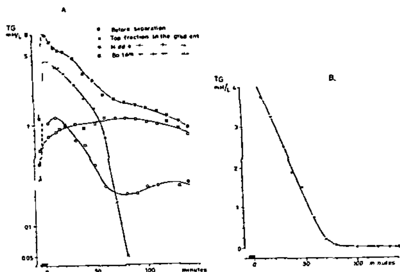


Fig 1 Elimination from the blood stream of intravenously injected chylomicrons (0 26 g fat/kg, case 1) The triglyceride concentration is given for plasma before and after separation in density gradients into 3 fractions (see methods) Dotted lines indicate extrapolation to zero time A Semilog scale B Top fraction on linear scale

15 hrs after the last meal and about 25 hrs after the cannulation procedure No reactions were observed from the patients during the infusion One of the patients (no 1) had a chill for 10 min, 3 hrs after the infusion and her body temperature rose 0 6° C above the morning temperature (37 7° C)

chylomicron samples were analysed as the plasma samples

linear graph (Hallberg 1964) A fractional removal rate (K_2 per min) was considered to be present when the curve was linear on a semilog graph The concentration at which K_1 changes into K_2 was defined as the "critical concentration" (C mmole/l) The analytical errors were calculated from the duplicate determinations according to the formula $S = \sqrt{\frac{\Sigma d^2}{2n}}$ (d = difference between duplicates and n = number of duplicates) The errors were

(n = 3[illegible])

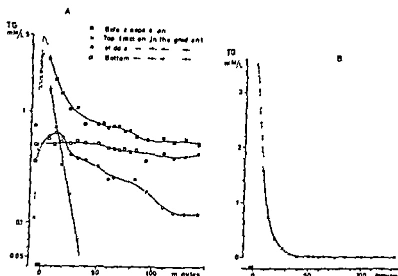


Fig 2 Elimination from the blood stream of intravenously injected chylomicrons (0 18 g fat/kg case 2) The triglyceride concentration is given for plasma before and after separation in density gradients into 3 fractions (see methods) Dotted lines indicate extrapolation to zero time A, Semilog scale B Top fraction on linear scale

Results

The volume and amount of TG in the infused chylomicron preparations are shown in Table I as well as the TG concentration in the different gradient fractions After separation of the chylomicron preparations in the density gradient 98 % and 97 % respectively of the TG present were found in the top fraction The middle fraction TG concentration in the lymph was somewhat higher than that found in the preinfusion plasma samples The bottom fraction TG concentration was about the same as that in the preinfusion plasma samples

The TG concentrations in plasma and its 3 gradient fractions before and after the infusions are set out in Fig 1 and 2 The top fraction TG concentration in case 1 *decreased apparently linearly at high concentrations and exponentially at low concentration* In case 2, the top fraction TG concentration decreased only exponentially The maximal elimination capacity (K_1) in case 1 was found to be 0 06 mmole/l/min The fractional removal rates (K_2) were 0 13 and 0 12 in the 2 studied cases

The middle fraction TG concentration showed a rapid increase followed by a decrease to a level below the preinfusion values The bottom fraction TG concentration in case 1 showed a slow increase which was followed by a slow decrease in case 2 there was an apparently unchanged level

Discussion

These two cases illustrate the same kinetic principle as that described earlier for the elimination of chylomicrons in dogs (Carlson and Hallberg 1963 and of an artificial fat emulsion in man (*cf* Hallberg 1965 d)

The "critical concentration", $C = K_1/K_2$ (*cf* Carlson and Hallberg 1963) was 0.5 mmole/l in case 1 and was not demonstrated in case 2. This concentration for the fat emulsion was shown to vary with the nutritional state (Hallberg 1965 c). Earlier observed ranges are 0.23 mmole/l to > 4.00 mmole/l (Hallberg 1965 a, c). The nutritional state of the subjects in this study was somewhat different from that of the subjects given the artificial fat emulsion: the latter had fasted for 15 hrs after an ordinary diet. The subjects in this study had fasted for 15 hrs after the intake of very high fat food on the day before the study and moreover, some of the dietary lipids were collected from the chyle. It was therefore difficult to assess the nutritional state of these subjects. At the moment there is no evidence that factors influencing the chylomicron elimination would be different from those of the tested artificial fat emulsion.

The origin of lipids in the chylomicrons is probably a factor of minor importance in the kinetic principle. In the dog experiments (Carlson and Hallberg 1963) the fat meal was a soyabean-oil emulsion, whereas in this study it consisted of cream from cow's milk.

The rate constants for the elimination of chylomicrons and the fat emulsion were of the same order of magnitude in dogs. The rate constants for the chylomicrons in this study were of the same order as those found for the fractional removal rate observed by Bierman and Hamlin 1962, and Nestle *et al.* 1962. The observed constants were also within the ranges found for the fat emulsion in man. The mean rate constants for the artificial emulsion was for K_1 0.07 mmole/l plasma/min (ranges 0.03 — > 0.40) and for K_2 0.06 per min (ranges 0.03—0.14) (Hallberg 1965 a, b, c).

The changes in TG concentration observed in the middle and bottom fractions during the elimination of fat emulsion were earlier interpreted as recirculating TG (Hallberg 1965 a, b, c). Such changes were also observed in this study. They were of the same nature (magnitude and duration) as those found after injection of the artificial fat emulsion. The amount of middle fraction TG in the infused chylomicron preparations was too small to be the sole explanation of the observed increase in plasma middle fraction TG.

This study thus showed that human chylomicrons were eliminated from the blood stream in the same way as the previously studied artificial fat emulsion, with regard to both the kinetic principle and the influence on endogenous plasma TG concentration.

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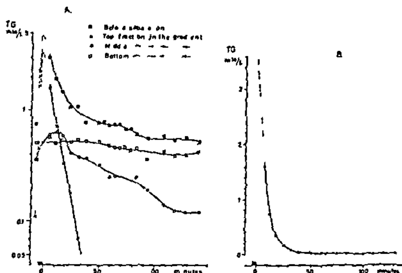


Fig 2 Elimination from the blood stream of intravenously injected chylomicrons (0 18 g fat/kg case 2) The triglyceride concentration is given for plasma before and after separation in density gradients into 3 fractions (see methods) Dotted lines indicate extrapolation to zero time A Semilog scale B Top fraction on linear scale

Results

The volume and amount of TG in the infused chylomicron preparations are shown in Table I, as well as the TG concentration in the different gradient fractions After separation of the chylomicron preparations in the density gradient 98 % and 97 % respectively of the TG present were found in the top fraction The middle fraction TG concentration in the lymph was somewhat higher than that found in the preinfusion plasma samples The bottom fraction TG concentration was about the same as that in the preinfusion plasma samples

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Discussion

These two cases illustrate the same kinetic principle as that described earlier for the elimination of chylomicrons in dogs (Carlson and Hallberg 1963) and of an artificial fat emulsion in man (*cf* Hallberg 1965 d)

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From the Department of Physiology Karolinska Institutet, Stockholm Sweden

Effect of Respiratory Acidosis on the Extraction of Noradrenaline in the Perfused Hindleg of Cats

By

S BYGDEMAN and L STJÄRNE

Respiratory acidosis and hypoxia has been shown to reduce the blood pressure increasing effect of intravascularly injected noradrenaline (NA) (Page and Olmsted 1951, Dunér and Euler 1959, Bygdeman 1963 Gowdey and Patel 1964 and others) However, concomitantly with a decreased effect of injected NA during hypoxia combined with hypercarbia the blood pressure response to carotid occlusion tests was normal or increased (Dunér and Euler 1959) Similar results have been obtained during periods of respiratory acidosis (Bygdeman 1963 Manley 1963)

It is conceivable that the failure of intravascularly injected NA to elicit a normal response during acidosis could be due to decreased penetration of the NA injected into the tissue fluid surrounding the vascular smooth muscle cells In view of this the effect of acidosis on the extraction of NA in a perfused hindleg preparation was studied in the present experiments using tritium labeled NA In order to find out whether a change in the NA extraction could be due to alteration in the transcapillary exchange of solutes the extraction of ^{86}Rb was also determined (Renkin and Rosell 1962)

[illegible]

about 40 min

[illegible]

p
p
0
p
2

intermittently for analysis of radioactivity

The cells were removed by centrifugation and 1 ml of 0.8 M perchloric acid was added to 1 ml of plasma After centrifugation 0.2 ml aliquots of the clear supernatant were counted in a 7 3 toluene-absolute ethanol solution, containing 4 g of 2 5-diphenyloxazole and 100 mg of 1 4 bis-2(5-phenyloxazolyl) benzene per liter of toluene using a Packard Liquid Scintillation Spectrometer

During the control periods before and after acidosis the extraction ratio

$$\left(\frac{\text{arterial } ^3\text{H NA} - \text{venous } ^3\text{H NA}}{\text{arterial } ^3\text{H NA}}\right)$$

for the ^{3}H NA was 44.2 $\pm$ 2.5 per cent and 39.6 $\pm$ 0.9 per cent respectively 3 expts The difference was not statistically significant (0.1 $>$ p $>$ 0.05 During acidosis the extraction ratio was reduced by 22 per cent to 32.7 $\pm$ 2.1 per cent the difference to the control periods being highly significant (0.005 $>$ p 0.001

The corresponding values for the extraction ratio for ^{86}Rb during the control periods were 53.8 1.4 per cent and 54.9 $\pm$ 2.1 per cent respectively 3 expts) Again the

extraction ratio during the acidosis period was reduced by 21 3 per cent, to 42 8 ± 2 0 per cent, (significantly lower than during the control periods $p < 0.001$)

The results of the present ^{86}Rb experiments clearly show that transcapillary exchange was reduced by acidosis Since the extraction ratio for ^{3}H dl NA fell by the same percentage, it may be concluded that this was probably due to a general decrease in transcapillary exchange of solutes, rather than to a selective change in the capillary permeability for NA

Thus the strong reduction in the vasoconstrictor response to i a injected NA during acidosis may be partly explained by decreased penetration of NA into the tissue fluid and therefore a lower NA concentration in this medium, available to trigger the receptors on the vascular smooth muscle cells

However, there are reasons to doubt that this could be the only, or even the most important reason Thus firstly, it seems unlikely that the relatively small reduction in the extraction ratio for NA during acidosis could explain the profound decrease in vasoconstrictor effect of injected NA Secondly, the vasoconstrictor effect of the sympathomimetic amines tyramine and phenylethylamine, which are believed to act by causing a local release of NA from the sympathetic nerve terminals (Burn and Rand 1958), was completely unchanged during acidosis (Bygdeman to be published) indicating that the impairment of amine transport in this case was of little importance

The mechanical reactivity of the vascular smooth muscle cells thus appears to be quite normal during acidosis Moreover no drastic change in the penetration of amines into the extravascular fluid could be demonstrated Thus the almost completely abolished response to i a injected NA in the presence of a normal response to nerve stimulation seems to be due to selective decrease in reactivity of the smooth muscle cell trigger mechanisms normally activated by circulating NA while the reactivity of trigger points normally activated by NA released from the sympathetic nerve terminals was unimpaired

Our results are compatible with the suggestion that locally released NA whether by nerve stimulation or pharmacologically may be protected by its very location in the narrow (200 Å) synaptic cleft , and that receptor mechanisms in this area may operate at a reasonably physiological pH at a time when the rest of the surface of the smooth muscle cells is severely damaged by the change in *e g* hydrogen ion concentration cf Bygdeman 1965)

Supported by a research grant to S B from The Swedish National Association f r Heart and Chest Diseases and a grant (Y 651 to L S from the Swedish Medical Research Council.

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Acta physiol. scand. 1965. 65. 287—288

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Osmiophilic Granules in Preaortal Paraganglia from Newborn Rabbits

By

TOMAS BRUNDIN and SVEN ERIK G NILSSON

In newborn mammals the catecholamine content of the preaortal paraganglia far exceeds that of the adrenal medulla (West 1955) It is well known that the catechol amines of the adrenal medulla are bound to subcellular osmiophilic granules (Hillarp, Lagerstedt and Nilson 1953), which occur in large numbers in the cytoplasm of adreno medullary cells (Lever 1955) However, no information seemed to be available on the ultramicroscopic structure of the paraganglia Recently it was found that high speed centrifugation sediments from homogenates of paraganglia contained > 70 % of the *total catecholamines of these organs (Brundin to be publ) This finding suggested that* also the paraganglionic catecholamines are bound to subcellular structures and prompted an electron microscopic study of the paraganglia

In the present study preaortal paraganglia and adrenals from newborn rabbits were fixed in situ with standard osmium tetroxide fixative which was dripped on to the organs The specimens were embedded in Vestopal W and the sections examined in an RCA EMU 3A electron microscope

An abundance of cytoplasmic granules were found in the paraganglionic cells (Fig 1, 2) The size of these granules, about 1,500 Å, corresponded approximately to that of the adrenomedullary ones (cf Fig 3) All the granules were membrane limited The adrenomedullary granules consisted of fairly large electron dense cores and less electron dense, dotted peripheries The paraganglionic granules showed considerably smaller electron dense cores the peripheral zones being large and of comparatively low opacity Although it can not be excluded that these morphological differences, consistently found in sections from several animals had occurred during the preparation procedure, the pictures suggest that the granules are of different kinds

It is highly probable that the osmiophilic granules demonstrated in the preaortal paraganglionic cells constitute the morphological basis for the storage of catecholamines in the paraganglia

This study has been supported by grants from Stiftelsen Lars Hiertas Minne and from Svenska Sällskapet för Medicinsk Forskning

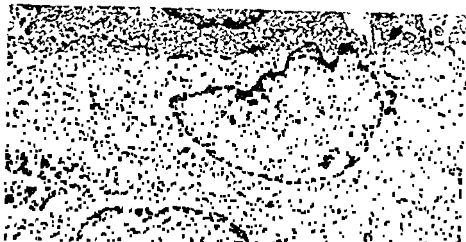


Fig 1 Survey picture of paraganglionic cells from a newborn rabbit 12,000 ×

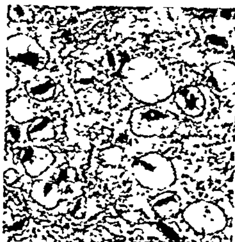


Fig 2

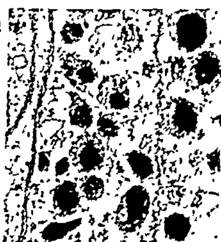


Fig 3

Fig 2 Osmiophilic granules in paraganglionic cells from the same animal as in Fig 1 55 000 ×

Fig 3 Osmiophilic granules in adrenomedullary cells from the same animal as in fig 1 2 55 000 ×

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Acta physiol. scand. 1965. 65. 289—291

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Influence of Dihydroergotamine and Adrenaline on the Concentrations of Glucose-6-Phosphate, Fructose-6-Phosphate, Adenosinetriphosphate and Creatinephosphate in Bovine Mesenteric Artery

By

A. Beviz and E. Mohme-Lundholm

Received 20 February 1965

Abstract

Beviz, A and E Mohme-Lundholm *Influence of dihydroergotamine and adrenaline on the concentrations of glucose-6-phosphate fructose-6-phosphate adenosinetriphosphate and creatinephosphate in bovine mesenteric artery* Acta physiol scand 1965 65 289—291 — In experiments on isolated mesenteric artery dihydroergotamine (DHE) at a concentration of $1 \cdot 10^{6}$ raised the adenosinetriphosphate (ATP) creatinephosphate (CrP) glucose-6-phosphate and fructose-6-phosphate levels in the muscle preparations On addition of adrenaline at a concentration of $1 \cdot 10^{6}$ following DHE the muscle relaxed coincidently with a further rise of the ATP CrP glucose 6-phosphate and fructose-6-phosphate contents Nethalide inhibited the relaxant effect of adrenaline In experiments on preparations not treated with DHE adrenaline contracted the muscle and reduced its ATP and CrP contents Adrenaline thus appears to have stimulated receptors of two types α receptors linked with arterial muscle contraction and hydrolysis of ATP and β receptors associated with relaxation synthesis of ATP and stimulation of carbohydrate metabolism

In previous experiments with isometric contraction of isolated mesenteric artery by adrenaline it was observed that the ATP and CrP contents decreased (Beviz *et al* 1965) and that the concentrations of certain intermediate carbohydrate metabolites such as glucose-6-phosphate, fructose-6-phosphate and fructose 1-6-diphosphate increased (Beviz and Mohme Lundholm 1964) The question was whether these changes in metabolite concentrations were direct results of the contractile process or whether adrenaline directly influenced the metabolism independently of that process — We have now explored this question by first blocking with dihydroergotamine DHE adrenaline s contractile action on vascular muscle then studying its effect on the ATP CrP glucose-6-phosphate and fructose-6 phosphate levels in the muscle

TABLE 1 *Effects of DHE at concentration of 10 ⁎ and adrenaline at concentration of 10 ⁎ on the glucose-6-phosphate fructose 6 phosphate, ATP and CrP contents of bovine mesenteric artery in μmoles/g wet weight Each value is the mean of ten tests P probability that the effect was due to chance*

	Glucose 6 *phosphate*	Fructose 6 *phosphate*	*ATP*	*CrP*
Basal values	*0 011 ± 0 0016*	0 007 ± 0 0015	0 63 ± 0 10	0 20 ± 0 01
Change from basal values after DHE	0 0074 ± 0 0026 P < 0 02	0 00 ± 0 001	0 13 ± 0 06 P < 0 05	0 011 ± 0 05
Change from DHE values after adrenaline	*0 013 ± 0 005* P < 0 02	*0 0064 ± 0 003* P < 0 05	0 13 ± 0 04 P < 0 01	0 017 ± 0 26

Methods

Results

Preparations treated with DHE showed a gradual rise of tension and an increase of the ATP glucose-6 phosphate and fructose-6 phosphate contents Table 1) Addition of adrenaline to such preparations caused a certain degree of relaxation Adrenaline raised the ATP glucose-6 phosphate and fructose 6 phosphate contents over and above the increase produced by DHE Nethalide at a concentration of 2 10 ⁎ added simultaneously with DHE blocked the relaxant effect of adrenaline

Discussion

The hypothesis was earlier advanced (Lundholm and Mohme-Lundholm 1963 that adrenaline stimulates the carbohydrate metabolism of vascular muscle via an direct mechanism The present findings lend support to that view

The contractile effect of adrenaline on smooth muscle is assumed to be mediated via stimulation of adrenergic *α receptors It appears likely that this alpha* effect was in these experiments, accompanied by hydrolysis of ATP and CrP There was a coincident increase in the content of intermediate carbohydrate metabolites When the α receptors were blocked with DHE adrenaline had a relaxant effect that could be inhibited with nethalide which *blocks β receptors* Hence, mesenteric arteries may well possess both *α* and *β receptors* In the presence of DHE, adrenaline raised the ATP level while still augmenting the glucose-6 phosphate and fructose-6-phosphate contents

Since nethalide has been found to inhibit the stimulatory effect of adrenaline on carbohydrate metabolism in vascular muscle (Lundholm and Mohme Lundholm 1965), the metabolic stimulation produced by adrenaline in vascular muscle might consist in an adrenergic β receptor effect

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From the Department of Physiology, University of Goteborg Sweden

Behavioural and Autonomic Patterns Evoked by Stimulation of the Lateral Hypothalamic Area in the Cat

By

BJORN FOLKOW and EDUARDO H RUBINSTEIN[1]

Received 1 March 1965

Abstract

Folkow B and E. H Rubinstein *Behavioural and autonomic patterns evoked by stimulation of the lateral hypothalamic area in the cat* Acta physiol scand 1965 65 292—299 — Topical electrical stimulation of the so-called feeding centre' and adjacent parts of the lateral hypothalamus of the cat was performed in awake animals In some of the animals a clearly stimulus-bound food consumption took place and stimulation of the same structure produced in the anesthetized animals a fairly specific autonomic adjustment involving activation of vagal fibres to the gastro-intestinal tract and a sympathetically mediated cardiovascular adjustment favouring gastro-intestinal blood flow The more frequently occurring behavioural response to stimulation of the lateral hypothalamic area involved a searching or appetitive exploratory response without any actual food intake during the stimulation When stimulation in the same electrode positions was performed in the anesthetized animals no consistent or clearcut autonomic adjustments could be observed The possible interrelationship between the feeding response" and the exploratory response' is briefly discussed These somatomotor and visceromotor response patterns differed markedly from those obtained by stimulation of more ventrally placed but closely adjacent parts of the lateral hypothalamus The effects induced from these electrode positions the alarm response" involved a well defined attack or flight reaction coupled with a sympathetically mediated gastro-intestinal inhibition and a characteristic cardiovascular adjustment favouring muscle blood flow at the expense of gastro-intestinal blood flow

The present ideas concerning the regulation of food intake focus on the central nervous system as playing the principal role in the control It is generally accepted that a lateral hypothalamic area the so-called feeding centre", can induce all the behavioural components related to the ingestion of food, when it reaches a certain level of excitability This hypothalamic area is constantly more or less under the inhibitory influence of a "satiety center" located at the level of the ventromedial hypothalamic nucleus whose excitability can also be modified by peripheral mechanisms

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This scheme, resulting from the work of different investigators and recently summarized by Anand (1961), is based principally on the effects of localized lesions in the described hypothalamic areas (aphagia from lateral hypothalamic lesions and hyperphagia from ventromedial hypothalamic lesions) Effects of electrical stimulation at the same brain localizations have, on the whole, largely confirmed the above mentioned hypothesis Apparently there may be some species differences as the alimentary reactions taking place during such stimulations are, for instance, more readily evoked in rats and goats than in cats, as described by Brugger (1943), Delgado and Anand (1953), Larsson (1954), Smith (1961), Miller (1961)

In the present investigation this alimentary response pattern, elicited upon hypothalamic stimulation in cats was studied with special regard to the possibility that a specific visceromotor pattern, adjusting gastro-intestinal function and blood flow, might be closely associated with the centrally evoked feeding response The feeding centre' is, however, located close to hypothalamic sites from where it is possible to induce alarm reactions (rage or flight), which are indeed associated with very marked autonomic changes It was therefore found justified to explore most parts of the lateral hypothalamic area in order to find out to what extent different behavioural expressions, integrated from these hypothalamic sections, are normally linked to distinct and well differentiated autonomic response patterns The preliminary results of this study have earlier been briefly reported (Folkow and Rubinstein 1963)

Material and methods

this level in most cats corresponded to the frontal plane 9 5—11 mm anterior to the interaural line Each multiple electrode ended in a miniature electronic tube socket that was attached to the occipital bone by stainless steel hooks and dental acrylic cement Stimulation tests were started two weeks after the operation The animals were placed in an isolation cage and their reactions observed through a one way window Square wave stimulating pulses of 100 cps 1 msec duration and variable peak current (0 05 to 0 7 mA) were delivered by a Grass S4C

anesthesia while several autonomic parameters were recorded Topical brain stimulations were now performed especially by way of those electrode contacts which to judge from the earlier behavioral studies induced characteristic response patterns in the awake animals Arterial blood pressure was measured from one of the femoral arteries by a mercury manometer and heart rate was estimated from the electrocardiogram Muscle and intestinal blood flows were recorded by diverting the venous outflow from the deep femoral and superior mesenteric veins respectively through closed optical drop-counters which operated ordinate writers Gastric and or intestinal motility was measured at constant pressure as changes in volume via Tyrode filled catheters which were placed in the visceral lumen and connected to piston recorders

After the acute experiments the brains were perfused with saline and 10 formalin and were then sectioned with a stereotaxically mounted knife to localize the electrode tracks Then each brain part containing electrode tracks was frozen sectioned in 50 μ slices and stained by the Nissl method to allow microscopic determination of the exact points of electrode stimulation

Results

I *Behavioural responses* Three types of clearcut modification of the behaviour of freely moving animals could be elicited by topical stimulation at different levels in the lateral parts of the hypothalamus A) *Feeding responses* In 3 cats a clear and reproducable sequence of acts was induced by stimulation via one of the electrode contacts With the cat lying but attentive in the cage, the topical stimulation was started The animal's level of awareness was raised and it rose, started to walk slowly in a searching like fashion while sniffing the floor, approached the food tray and began to eat This whole series of events in these cats was regularly completed before the end of the stimulating period of 10 sec

B) *Exploratory responses* In most of the animals topical stimulation via some of the electrode contacts brought about only part of the food-oriented behaviour, namely *the searching activity A slow, attentive exploration of the cage was then induced,* apparently without any affective component', the animal looking into the food tray but without starting to eat at least not in direct connection with the stimulation

C) *Alarm responses* Two types of behavioural responses are here considered viz attack (rage") and flight (fear") Both reactions were gradually built up during the stimulation and could be greatly reinforced if the strength of the stimulation was slightly raised The attack reaction was initially characterized mainly by arousal and mydriasis but within a few seconds growling hissing, spitting arching the back piloerection and protrusion of the claws ensued and finally a violent attack followed associated with hyperventilation, salivation and urination The flight reaction too began with arousal but soon the animal started a fast exploration of the cage looked frequently towards the trailing stimulating wire rose repeatedly on its hindlimbs towards the walls of the cage as if looking for a way of escape and finally jumped high up towards the roof of the cage the only apparent way of escape

There was a very clear difference between the arousal and exploration behaviour described in B and in C The behaviour described in B) was a non affective type of searching activity swiftly conducted and relatively unaffected by interfering auditory or visual stimuli while the behaviour in C was characterized by a fast activity which could often be triggered to its final crescendo in the form of an attack or escape reaction by a trivial auditory or visual stimulation like tapping the walls of the cage

There were no consistent behavioural effects after the stimulus was turned off Occasional licking or cleaning was observed resembling to some extent the displaced activities described by ethological workers Thorpe 1956

II *Autonomic responses* Two distinct patterns of autonomic responses were consistently recorded when stimulation of the electrode contacts which yielded feeding A) or alarm (C responses was performed in the lightly anesthetized animal

"*Feeding pattern* Fig 1 illustrates a characteristic effect of topical stimulation at points which induced clearcut and reproducable feeding responses in the awake animal These stimulations induced slight increases in blood pressure and in heart rate a marked increase in intestinal motility and a slight increase in intestinal blood flow while muscle blood flow was moderately reduced

The increased intestinal motility was evidently mediated via the vagal nerves as it was abolished by vagal section or by atropine This intestinal excitation was accompanied by a weak increase in gastric motility if the stomach to start with was distended On the other hand when in one of the experiments gastric volume was recorded in

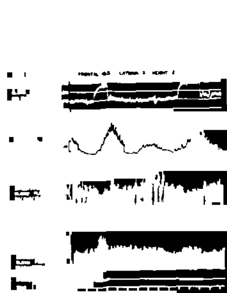


Fig 1

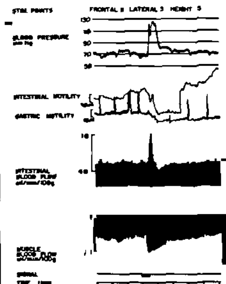


Fig 2

Fig 1 Cat 2.5 kg Chloralose Autonomic effects of stimulation of an electrode which in the awake animal elicited a fully developed feeding response Note the moderate blood pressure increase and the reduced muscle blood flow combined with an increased intestinal blood flow and motility

Fig 2 Cat 2 8 kg Chloralose Autonomic effects induced by an electrode which in the awake animal caused an alarm response (attack) Note that in this case the rather sharp blood pressure increase is combined with a considerable increase in muscle blood flow while intestinal blood flow is promptly reduced and intestinal and gastric motility inhibited

such a way that gastric transmural pressure was kept at a low but constant level (see Jansson and Martinson 1965) a prompt and considerable volume increase that was abolished by vagotomy could be repeatedly induced by topical stimulations in the 'feeding' area This complex sympathetic and parasympathetic pattern was as mentioned obviously only in those animals which in the awake state showed a typical feeding response to the same topical stimulation

In contrast stimulation at those electrode sites which in the awake animal produced the exploratory reaction (B) elicited no clearcut and consistent autonomic adjustments in the lightly anesthetized preparations

Rage pattern In 8 cats where topical stimulation induced clearcut rage or flight reactions in the awake animal the characteristic autonomic defence reaction cf Abrahams *et al* 1960 was obtained during the acute experiments in light anesthesia As illustrated in Fig 2 such stimulation elicited an increase in blood pressure an inhibition of the prevailing intestinal and gastric motility a pronounced reduction in intestinal blood flow and an increased muscle blood flow The muscle blood flow increase was largely abolished by atropine suggesting that it was dependent on an activa

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autonomic adjustment of gastro-intestinal function and blood flow distribution For such reasons a careful study of the behaviour of awake animals, exposed to topical stimulations in the lateral hypothalamic area, was first performed In all the cats where the feeding centre and or its immediate surroundings was electrically stimulated it was observed that a characteristic "exploratory response' took place, but only in a limited number of cases did the electrically induced searching activity result regularly and promptly in food consumption

The autonomic changes elicited from the same location which induced the fully developed feeding response, were characterized by in extent relatively moderate vasomotor adjustments while the vagal excitation of intestinal motility appeared to be more intense As compared to the intestinal responses it was difficult to reveal to what extent and in what direction the gastric smooth muscles were affected, but presumably this was at least partly due to the technique utilized for recording gastric motility In two of these experiments the stomach was initially filled to distension and it exhibited a low spontaneous activity Hypothalamic stimulation then induced what appeared to be a slight motility increase However, when in the third of these experiments the stomach was initially only slightly filled at a low constant transmural pressure and

carry 'inhibitory' fibres (Martinson and Muren 1963) which under optimal conditions can produce a profound relaxation of the corpus-fundus regions (Jansson and Martinson 1965) The influence of these latter fibres is however clearly revealed only if the stomach to start with is small and not distended by any high transmural pressure The two sets of efferent vagal fibres by no means seem to be truly antagonistic but rather appear to be synergistic In fact when the corpus fundus region becomes relaxed by the vagal inhibitory vagal fibres the influence of the "excitatory fibres is greatly enhanced so the net result of activating them both will be an increased motility at an increased average lumen of the reservoir organ (Jansson and Martinson 1965) The different types of reactions of the stomach to hypothalamic stimulation in these experi-

was initially small and exposed to a low but constant distending pressure — a situation favourable for revealing the influence of the inhibitory vagal fibres — it dilated in response to the hypothalamic stimulation It is not unlikely that both types of vagal fibres were in fact activated from the hypothalamic feeding centre creating a sort of anticipatory receptive relaxation combined with increased motility of the wall More studies of the gastric responses are needed however before this question can be definitely settled the present observations are with respect to the gastric reactions preliminary

In any case it is well known from many earlier studies that gastro-intestinal motility changes can be induced by hypothalamic stimulation Beattie and Sheehan 1934 Kabat *et al* 1935 Strom and Uvnas 1950 Eliasson 1954 In these earlier studies the gastric responses have often been difficult to interpret but the recent finding that there is another group of efferent vagal fibres which produce relaxation and widening of the corpus-fundus region Jansson and Martinson 1963 may explain as mentioned not only the present observations but also some of the earlier results concerning gastric

responses to brain stimulations The present data are in general agreement with the above-mentioned earlier studies and in addition the gastro-intestinal reactions studied here, seem to be closely correlated with and integrated by the feeding centre, which on stimulation in the awake cat produced food intake

The neurogenic adjustments of the cardiovascular system observed in connection with stimulation of the feeding centre were not drastic in extent, but decidedly of such a type as to favour the blood supply to the gastro-intestinal tract at the expense of that of the skeletal muscles The changes observed are consistent with the idea that a moderate increase in sympathetic adrenergic discharge to the cardiovascular system takes place, with little or no involvement of the vasoconstrictor fibres to the gastrointestinal tract where, instead, the constrictor fibre activity may become inhibited, possibly via the baroreceptors As specific vagal vasodilator fibres to the intestine do not exist, to judge from a recent study (Kewenter 1965) and, moreover, since vagotomy did not significantly affect the vascular adjustments obtained, the intestinal blood flow increase cannot be ascribed to any vagal influence Vasomotor fibre responses of this general character were occasionally observed by Lofving in studies where the autonomic structures in the limbic system were stimulated (see e g Fig 4 D, Löfving 1961)

These results in anesthetized animals cannot be exactly compared with the cardiovascular changes observed in awake dogs when food was presented (Rushmer *et al* 1960), even if there is no disagreement with these findings In this latter study, apart from the species difference, the animals were awake while the cardiovascular responses were recorded and they were able to move around, adding to some extent an adjustment of the circulation, caused by the movements and possibly by mental excitation When presented with food, dogs usually show a clear orienting reaction that by itself seems to induce prompt cardiovascular adjustments, apparent, for instance as a tachycardia (Robinson and Gantt 1947)

The "exploratory response without actual food consumption which was observed upon stimulation of hypothalamic locations overlapping or surrounding the area which initiated the fully developed feeding response" may suggest that this latter area is normally overlapped by another perhaps bigger area related to a fairly non affective exploratory or appetitive activity This latter behavioural pattern was evidently not connected to any distinct autonomic adjustments to judge from the results of the acute experiments Thus characteristic autonomic adjustments were obtained only when electrode points eliciting the fully developed feeding response were stimulated. It may be so that these autonomic adjustments especially the gastro-intestinal changes serve as an additional facilitatory influence by eliciting afferent impulses from the gastro-intestinal tract helping to direct the animal towards food and to initiate consumption

The anatomical localization the behavioural responses and the autonomic effects characterizing the pattern here called the alarm response are in full agreement with a considerable amount of earlier observations recently summarized by Mets 1961 starting with the classic description by Hess 1928 of the affective defensive reaction in the awake cat This alarm response which was induced from more ventral electrode positions in the lateral hypothalamic area was here studied mainly to underline the drastic differences in type and direction of the autonomic neurogenic adjustments, forming part of reactions so different in nature as those involved in alarm and feeding responses These effects were markedly different even when elicited from closely situated hypothalamic sites and shifting the stimulation point only 1 1 5 mm could

change the one response pattern to the other It is therefore unlikely that some of the observed autonomic adjustments should merely be expressions of a current spread to some common excitable region

This study was supported by grants from the Swedish Medical Research Council (grant Y 118) from the School of Aerospace Medicine AFSC through the European Office Aerospace Research United States Air Force (AF 61(052) 732) and from the U S Public Health Service (HE-05675 03-04)

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The Effect of Graded Vagal Stimulation on Gastric Motility, Secretion and Blood Flow in the Cat

By

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Abstract

Martinsson J. *The effect of graded vagal stimulation on gastric motility secretion and blood flow in the ca[t]* Acta physiol scand 1965 *65* 300—309 — In experiments on cats gastric motility secretion and blood flow were studied simultaneously or separately. By applying efferent vagal stimulation [illegible]

fibre group with a considerably higher stimulation threshold than the fibre group that enhances gastric motility. Both fibre groups can exert their control at impulse frequencies below 10 imp/sec. It is possible that secretion of both HCl and pepsin, relaxation and vasodilatation normally form a gastric response pattern elicited by a concomitant excitation of the efferent high threshold fibres of the vagus nerves

It is generally recognized that the vagal control of gastrointestinal functions is accomplished by means of a relatively small number of efferent nerve fibres. Thus the abdominal vagus nerves of the cat are made up of only about 3 000 efferent fibres which is less than 10 % of the total number of their fibres (Agostoni *et al* 1957). This small group of fibres controls various motor and secretory functions of the gastrointestinal tract. Even the small total number of fibres in the vagus nerves implies a considerable extent of divergence in the contacts with the far greater number of cells in the intrinsic nerve plexa. The question also arises whether a single efferent vagal fibre can control more than one functional parameter in a given gastrointestinal response pattern such as both secretory and motor effects.

The present study was prompted mainly by Martinson's and Muren's (1963) observation that gastric motility is controlled by two groups of fibres, a low threshold type eliciting excitatory responses from the stomach and a high threshold type inhibiting motility. Fibres of the latter type have proved extremely potent in augmenting gastric volume (Jansson and Martinson 1965). Furthermore the relaxing fibres have been

shown to be non-adrenergic (Martinson 1965), though the relaxing effect is not blocked by atropine

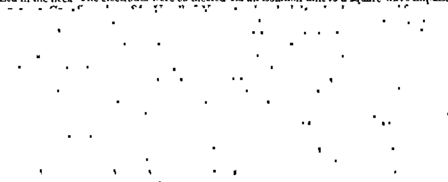
It was soon realized that gastric secretion was not controlled by such an arrangement of excitatory and inhibitory vagal fibres The present study was therefore undertaken to compare the responses of various functions of the stomach to graded vagal stimuli The gastric functions studied were secretion, motility and blood flow

Material and methods

50 cats weighing between 1 4 and 3 6 kg were used Anesthesia was induced with ether followed by an i v injection of chloralose and urethane (50 and 80 mg/kg respectively), while in the later two thirds of experiments only chloralose, 70 mg/kg, was used. A tracheal cannula was inserted to maintain the air passage

The abdominal cavity was opened by a midline incision In a few experiments in which the gastric blood flow was recorded *via* the portal vein the intestines and the pancreas was removed A ligature was placed around the muscular coat of the lower oesophagus, care being taken not to damage the vagus nerves

Stimulation of vagus nerves In all experiments the cervical vagus nerves were dissected free and divided When these nerves were to be stimulated their peripheral ends were placed in two bipolar electrodes with 2 5 mm between the annular silver poles When the stimulation was performed intra abdominally that segment of the anterior vagus nerve trunk below the diaphragm was dissected free divided and placed in a stimulation electrode similar to that used in the neck The electrodes were connected *via* an isolation unit to a square wave impulse



The temperature of the saline was kept at 33° C

Motility was studied with the balloon technique in 16 expts and with volumetric recordings in 21 expts.

Gastric secretion Secretion was studied in 28 expts The stomach was prepared in essentially the same way as described above 10—20 ml of isotonic saline, warmed to 38° C was injected into the stomach at regular intervals of 15 or 20 min At the end of each interval the fluid was withdrawn and its volume was measured Sometimes shorter intervals (3—5 min) were used for 3—4 collections immediately after a stimulation period in order to remove gastric juice produced and to allow subsequent estimation of the poststimulatory basal secretion A fraction of the gastric juice sample was pipetted off and kept in the frozen state until determination of its pepsin content on the following day The concentration of hydrochloric acid in the sample was measured by titration with 0 05 N NaOH using phenolphthalein as an indicator Peptic activity was determined by a slight modification of the method described by Hunt (1948 A dried human plasma protein preparation devoid of the γ-globulin fraction (Fraction IVb kindly supplied by AB KABI Stockholm or human excess plasma acidified with 3—5 N HCl to a pH of 2 1 was used as substrate The extinction values were read in a spectrophotometer at 720 mμ The accuracy of the method was tested by making double analyses and by analyses of various dilutions of gastric juice samples and was found to be about ± 10 % or considerably better

when the pepsin concentration exceeded 10—15 PU/ml Plotting of the observed pepsin concentrations of the different diluted samples against the degree of dilution gave a linear correla-

mesenteric vein after exclusion of the entire intestine, the omentum, spleen and pancreas

i e about one third of the stomach However, the simplest procedure was to collect the venous outflow from the central end of splenic vein at its union with the superior mesenteric vein

in the major part of the pancreas

The venous outflow was returned to the animal *via* one of the jugular veins Heparin (5 mg/kg) was given i v shortly before the blood flow recording cannulas were inserted The drop chamber and tubing were primed with dextrane and occasional blood losses during the

peripheral resistance was calculated as an approximate

$$\mathrm{PRU}_{100} = \frac{\text{Mean arterial pressure in mm Hg}}{\text{Blood flow in ml/min} \times 100\ \text{g tissue}}$$

This value includes the resistance of the drop-counting device and its tubing which was ver

and blood

he remain

ing 24 expts satisfactory recordings were obtained for only one of these three functions

All values on deviation given together with mean values are standard error of means

Results

Basal conditions The pressure within the stomach as measured with the balloon technique varied considerably but was usually between 5 and 10 cm of water small rhythmic waves which were not synchronous with respiration were also recorded According to the fluid-filled volume-recording system, the stomach contained a volume of about 10 20 ml as measured at a constant intraluminal pressure of 2 5 4 cm water Basal secretion of hydrochloric acid was 0 04 ± 0 002 meq 15 min pepsin secretion was about 50 ± 30 pepsin units 15 min Resting blood flow 18 ± 1 5 ml min and 100 g of tissue Vascular peripheral resistance, PRU_{100} ranged between 14 and 2 8 mean 5 5 Blood flow often remained constant even during substantial changes in arterial pressure Mean arterial blood pressure averaged 100 ± 6 mm Hg

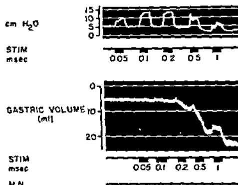


Fig 1 Cat 2 6 kg Gastric motility recorded by two different methods during bilateral cervical vagal stimulation 8 imp/sec 5 V impulse duration varied
Upper intraluminal pressure recorded with balloon technique Note slight after relaxation and depression of response at stimulation above 0.2 msec
Lower gastric volume recording at intraluminal pressure of 3 cm H O Note absence of excitatory responses but after relaxations clearly visible at same impulse durations as in the upper recording
Time separating the both recordings about 30 min

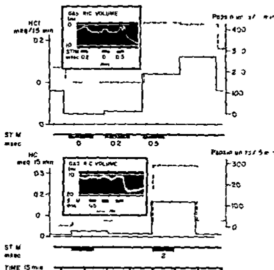


Fig 2 Gastric secretion and motility during vagal stimulation Bilateral cervical stimulation 4 imp/sec 5 V Impulse duration varied (msec) Motility recorded with "volume technique (see text) HCl (———) Pepsin ( )
Upper Cat 3 4 kg Ordinary threshold level (0 5 msec) both for secretion and relaxation
Lower Cat 2 4 kg Nerve preparation which needed higher duration values (see text)

Vagal stimulation All functions of the stomach studied were affected by vagal stimulation The less satisfactory reproducibility of the responses of the blood flow and secretion limited the value of quantitative analyses The responses to equal stimuli usually tended to decrease successively during an experiment In order to eliminate the possible source of error induced by such presumably unspecific influences the order in which the various impulse frequencies were used in consecutive periods of stimulation was varied at random (Fig 3 The strength of the response did not differ significantly with the method of stimulation left right bilateral cervical intra abdominal

In order to ascertain the stimulation thresholds of different fibres in the vagus nerves the pulse duration was varied voltage and frequency were kept constant at 5 volts and 4 or 8 imp sec In 8 of 16 expts using these conditions and the balloon technique the stomach responded with a clear increase of gastric tone at pulse duration 0 05 msec

when the pepsin concentration exceeded 10—15 PU/ml Plotting of the observed pepsin con centrations of the different diluted samples against the degree of dilution gave a linear correla-

flow from the stomach through a drop-chamber, in which the drops were photoelectrically counted and operated an ordinate writer Venous outflow was collected in different ways In the beginning of the investigation venous outflow was collected in the portal vein or in the superior mesenteric vein after exclusion of the entire intestine, the omentum, spleen and pancreas. Sometimes, however, this method proved less satisfactory possibly due to the exclusion of

flow measured came mainly from the minor curvature of the corpus fundus and from the antrum i e about one third of the stomach However, the simplest procedure was to collect the venous outflow from the central end of splenic vein at its union with the superior mesenteric vein The right gastric and right gastroepiploic veins emptying directly into the portal vein were then ligated The other organs drained by the splenic vein i e the spleen, the greater omentum and the tail of the pancreas were removed With this technique the portal blood flow from the rest of the intestinal canal was not impaired It also made it possible to preserve the circulation in the major part of the pancreas

The venous outflow was returned to the animal via one of the jugular veins Heparin (5 mg/kg) was given i v shortly before the blood flow-recording cannulas were inserted The drop chamber and tubing were primed with dextrane and occasional blood losses during the experiment were substituted by dextrane usually at most 10 ml, with due control of the hema tocrit In all but 2 expts the sympathetic supply was left intact In the other experiments the left major splanchnic nerve was cut for stimulation (results not reported here)

The blood pressure in one femoral artery was recorded by a mercury manometer Vascular peripheral resistance was calculated as an approximate

$$PRU_{100} = \frac{\text{Mean arterial pressure in mm Hg}}{\text{Blood flow in ml/min} \times 100 \text{ g tissue}},$$

ch was ref

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All values on deviation given together with mean values are standard error of means

Results

Basal conditions The pressure within the stomach as measured with the balloon technique varied considerably but was usually between 5 and 10 cm of water small rhythmic waves which were not synchronous with respiration were also recorded According to the fluid filled volume-recording system the stomach contained a volume of about 10—20 ml, as measured at a constant intraluminal pressure of 2 5—4 cm water Basal secretion of hydrochloric acid was 0 04 ± 0 002 meq 15 min pepsin secretion was about 50 ± 30 pepsin units 15 min Resting blood flow 18 ± 1 5 ml min and 100 g of tissue, Vascular peripheral resistance PRU$_{100}$ ranged between 14 and 28 (mean 5 5) Blood flow often remained constant even during substantial changes in arterial pressure Mean arterial blood pressure averaged 100 ± 6 mm Hg

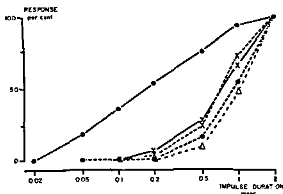


Fig 5 Mean gastric responses to vagal stimulation with 8 imp/sec, 5 V, and pulse duration varied according to the abscissa

●——● Excitatory motor responses (balloon technique n = 15) per cent of maximal excitatory response
●- - ● Relaxation responses(volume recording), n = 11
×——× Secretion of HCl, n = 8
×- - - -× Secretion of pepsin, n = 7
△- - - △ Vasodilatation, n = 7

(Relaxation, HCl, pepsin and vasodilatation: Expressed as per cent of response at 2 msec)

same animal, simultaneously (Fig 3 and 4) or in succession (Fig 1 and 2) secretion of both HCl and pepsin, vasodilatation and relaxation appeared at the same stimula-

secretion and relaxation was 2 msec The different gastric responses to the threshold level stimulation varied considerably from being barely discernible to nearly maximal compared with the responses to stimulation with pulses of longer durations In Fig 5 the mean gastric responses to vagal stimulation are given at the different durations of the pulses This diagram is based on a number of stimulation series complete up to 2 msec, pulse frequency 8 imp/sec and 5 V (number of experiments for each function studied are given in the figure legend) Motor excitatory responses are expressed as per cent of maximal response and the inhibitory responses are excluded The responses in the high-threshold group were all expressed as per cent of the response to 2 msec, because it was not always possible to determine the maximal response

In a few experiments similar tests with varying pulse duration were made also at 10, and 2 V, respectively The general outcome of such experiments was that the stimulation threshold varied along a strength-duration curve of the shape described by Martinson and Muren (1963), *i e*, at the average threshold level at 5 V 0 5 msec corresponded to 0 1 or 0 2 msec at 10 V and 2 or 5 msec at 2 V for all the gastric functions studied

Most of the above results were obtained with a stimulus-impulse frequency of 8 imp/sec, which invariably elicited the maximal secretory response The average amount of hydrochloric acid secreted at 8 imp/sec (1—5 msec, 5V) was 0 45 ± 0 06 meq/15 min of stimulation (n = 17) and 2500 ± 900 pepsin units/15 min of stimulation (n = 15) The responses to 4 imp/sec and to 16 imp/sec were both smaller than the response to 8 imp sec, irrespective of the order in which the different frequencies were

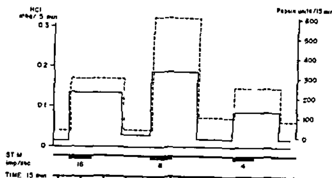


Fig 6 Cat 2.4 kg Gastric secretion recorded at bilateral cervical vagal stimulation 5 V, 5 msec, frequency varied HCl secretion rate (———), Pepsin (- - - - - -)

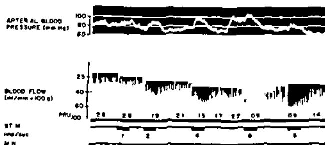


Fig 7 Cat 2.9 kg Blood flow recording at intra-abdominal vagal stimulation 5 V, 2 msec, frequency varied As regards pressor responses to vagal stimulation *cf* Fig 4

tested (Fig 6) The mean response to 4 imp/sec (n = 9) was 0.32 ± 0.04 meq and 1600 ± 250 pepsin units/15 min of stimulation At 16 imp/sec (n = 8) the responses were 0.27 ± 0.03 meq and 1400 ± 500 pepsin units/15 min of stimulation

Although it was not possible to obtain a closer frequency-response correlation the above findings are compatible with an optimal stimulation frequency *around 8 imp/sec* and definitely below 16 imp/sec

Similarly, the vasodilatation observed could not be enhanced by increasing the impulse frequency above 8 imp/sec (Fig 7) Sometimes the blood flow was initially very high during stimulation with 16 or more imp/sec The flow afterwards gradually decreased and levelled off at a lower blood flow than that obtained at 8 imp/sec

Fig 7 shows the experiment with the highest initial blood flow (32 ml/min × 100 g of tissue) and a blood flow of 85 ml/min × 100 g during maximal vagal activation The mean blood flow during vagal stimulation with 8 imp/sec (1—5 msec and 5 V) was 43 ± ± 6 ml/min × 100 g (n = 13) The maximal blood flow that could be induced by intra-arterial infusion of isopropylnoradrenaline sulphate was *100—120 ml/min* × 100 g of tissue (PRU_{100} = 0.7)

The quantitative correlation between blood flow and secretion was not studied in detail It was, however, *noted in one experiment that at a blood flow* as low as only

8 ml/(min × 100 g tissue) the stomach still secreted about 0 18 meq and 600 pepsin units/15 min of vagal stimulation (8 imp/sec, 5 V, 2 msec) On the other hand, in an experiment where the blood flow was 44 ml/min × 100 g during high threshold stimulation the secretion response was 0 49 meq and 1900 pepsin units/15 min of stimulation Another property of the neuroeffector systems of the stomach which however falls beyond the scope of this investigation was the curious extremely long latency between onset of vagal stimulation and recording of any secretory response (for ref see Gregory 1962, p 17 ff) In 3 expts with short sampling intervals (5 min) both pepsin and HCl secretion had clearly increased during the second 5 min period of stimulation i e a response was usually detectable with a latency of 5 to 10 min This latency probably holds only for the secretion of water and hydrochloric acid The pepsinogen presumably had to be washed out from the crypts of the gastric mucosa by the oxyntic cell secretion and therefore the observed latency was the same also for the pepsinogen secretion However, to check whether a response to low threshold stimulations would have occurred if the stimulation had been prolonged beyond 15 min 3 expts were performed with a constant low threshold stimulation (0 1 msec 5 V) throughout the experiment (i e for 3—5 hrs) In these cases the basal secretion was not elevated and remained constant When the constant low threshold stimulation at times was changed for the usual periods of graded stimuli the stomach responded with secretion at the expected levels

Discussion

The different effects obtained on graded vagal stimulation suggest that the vagus nerves contain two different fibre groups transmitting efferent impulses to the stomach In the following these two fibre groups are called "low threshold and high threshold" fibres, although both groups after all belong to high threshold" nerve fibres of the body Though it is not known whether this also means that they are made up of fibres of distinctly different diameters they differ so distinctly in threshold level that they can be differentiated by graded stimulation

The average stimulation thresholds for both excitatory and inhibitory motor fibres were shifted towards lower values as compared with the thresholds at 5 V reported by Martinson and Muren (1963) The probable explanation for this is that more efficient stimulation electrodes were used in the present experiments As seen from the results the stimulation thresholds varied also in this series of experiments (e g Fig 2) This is regarded as mainly a difference in resistance of the nerve preparation placed in the electrodes and not primarily as a difference between the nerve fibres in one cat compared with another The low threshold fibres only rarely needed more than 0 1 msec to be activated (at 5 V)and the high threshold fibres in almost all cases were activated at 0 5 or 1 msec This indicates similar stimulation conditions in different animals

When stimulated the high threshold group of fibres elicit a peripheral response pattern whose individual responses cannot be separated by means of the electrophysiological properties of the nerve fibres The results of experiments using different stimulation frequencies suggest that the high threshold fibre group exert its full range of control at an impulse discharge rate of less than 10 imp sec (cf Martinson 1964

It is tempting to imagine that the three responses secretion vasodilatation and relaxation are elicited in combination also in normally functioning stomach Such an organisation would easily fit in with the physiological functions of the stomach when the cephalic

phase of digestion is induced, secretion of a gastric juice rich in acid and pepsin starts. This is presumably a high energy consuming process (Crane and Davies 1951) requiring increased blood flow (functional vasodilatation) Also a neurogenic change in gastric muscle tone prepares the stomach in particular the corpus and fundus for the receptive relaxation Certain observations indicate that these responses in fact occur simultaneously Lorber, Komarov and Shay (1950) and Olbe and Jacobson (1963) and others have observed an inhibition of the motility of the stomach during sham feeding increasing acid secretion Further comparisons between these observations and the relaxation of the stomach seen in the present study must, however, be made with caution for in most studies the gastric motility inhibited during sham feeding was recorded in the antrum part, and Lorber *et al* found increased tone but inhibited rhythmic movements of the fundus Jansson and Martinson (1965) reported the relaxation to take place in the corpus fundus section, whereas the motility of the antrum was enhanced

Vagovagal reflexes elicited by stimulation of afferent nerve fibres presumably arising from mechano- and chemoreceptors in the stomach have been shown to elicit long lasting gastric relaxation and excitatory responses in most other gastrointestinal effectors, including the hydrochloric acid and pepsinogen producing cells (Harper Kidd and Scratcherd 1959)

In contrast to what is seen during sham feeding Lorber and Shay (1962) found that insulin hypoglycemia increased *not* only the secretion response but also the motility of the innervated gastric pouch as measured by means of a pressure recording device However, this finding does not exclude the possibility that the vagal inhibitory fibres were also excited As a matter of fact Jögi, Ström and Uvnas (1949) found a substantial initial inhibition of gastric motility after insulin Insulin hypoglycemia evidently stimulates the vagal excitatory fibres to the smooth muscles of the stomach and co-excitation of the inhibitory fibres can then be clearly revealed by a volumetric but not by pressure recordings (*cf* Jansson and Martinson 1965)

Most stimuli inducing gastric secretion also increase blood flow through the active mucosa (Jacobson 1963) Wolf and Wolff (1943 p 129 ff) showed that this occurs also when the stimuli are apparently purely nervous It remains to be shown whether this vasodilatation is at least in part due to specific vasodilator nerves or entirely to metabolites from the secreting glands Any such evidence must also take into account the specific plasma kinin mechanism possibly involved in the functional vasodilatation of the salivary glands (Hilton and Lewis 1956)

No increase in gastric blood flow on vagal stimulation was observed by Burton Opitz (1910 Lim Necheles and Ni (1927) or Boenheim 1930) However for several reasons it seems as if their results must be ascribed to the fact that the vascular bed of the stomach was already initially strongly dilated For instance for technical reasons Burton Opitz had to work during a short period of reactive hyperemia and Boenheim used large doses of chloral hydrate as anesthesia a substance known to markedly depress basal vascular tone Under such conditions it must surely be almost impossible to demonstrate the existence of any functional vasodilatation

It appears that the smooth muscles of the stomach do not require an increased blood flow during vagal activation Fig 2 left panel) This also seems to hold for the intestine for even intense vagal excitation of the intestinal smooth muscle will not increase intestinal blood flow Kewenter 1965

The fewness of the efferent nerve fibres in the vagus nerves Agostoni *et al* 1957 might also justify the assumption that the high threshold group of efferent fibres controls

a peripheral response pattern, delicately governed only by the far more numerous local nerve cells in the intrinsic plexa, and possibly also by local hormones Certain pharmacological and other differences between the various neuroeffector systems, such as atropine sensitivity (*cf* Martinson 1965) and the long latency for HCl secretion are presumably to be found at this level or in the effector cells

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The Effect of Sustained and Rhythmic Contractions on the Electromyogram (EMG)

By

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Abstract

[illegible] and rhythmic contraction [illegible] MG [illegible] ted [illegible] ms [illegible] f a duration, the sum of which was equal to the duration of the sustained contractions *Particularly* [illegible] contractions or [illegible] of polyphasic [illegible] ntials decreased [illegible] EMG changes are discussed particularly with regard to the circulatory conditions and metabolism of the muscle in the various types of muscle contractions

Various changes have been described to occur in the electromyogram during and after muscular exercise Thus several authors (for references see Scherrer and Monod 1960) state that the amplitude of the electromyographic action potentials decrease in exercising muscles. Buchthal *et al* (1951 1954 b) have observed an increased number of polyphasic action potentials in normal subjects occuring after heavy muscular work but no statistically significant change in the duration and amplitude of the action potentials. An increased number of polyphasic action potentials and a decreased duration of the action potentials were also observed after exercise in patients with a certain type of hereditary *paroxysmal myoglobinuria* (Larsson *et al* 1964) The EMG changes in these patients appeared even after a slight exercise on a bicycle ergometer while similar EMG changes, but less pronounced appeared in normal subjects only after heavy exercise The purpose of the present investigation was to examine changes in the EMG of normal subjects after various types of standardized muscular work

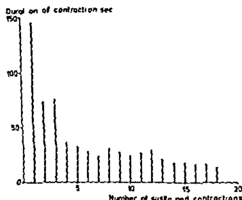


Fig 1 Duration of sustained contractions of elbow flexor muscles in a typical experiment

Material

Twenty healthy volunteers, 4 females and 16 males, 20—40 (median 24) years of age were examined They had stated that they were willing to perform maximal work

Methods

EMG was recorded before and after various types of standardized exercise Before the work the recordings were made on the subjects after their arrival to the laboratory from their daily activities 15—20 min after the end of the standardized exercise the recordings started again and continued for about 20 min

Dynamic rhythmic muscular work on a bicycle ergometer Work was performed with either legs or arms on a bicycle ergometer (Holmgren and Mattson 1954) The subjects worked for 6 min periods and the load was increased stepwise with 200 or 300 kpm/min until the work intensity became intolerable i e to a work intensity that was as far as can be judged maximal Electrocardiogram and respiratory frequency were recorded during exercise

Sustained isometric muscle contractions of maximal duration The subject stood upright sustaining the elbow by the hip bone with the elbow flexed about 90° A 10 kg weight hanging in a leather strap was held in the hand The load corresponded to about 1/3 of the maximal voluntary force of contraction of the elbow flexors The subject was instructed and encouraged to keep the weight as long as possible The contraction of the muscle was interrupted when the subject was unable to maintain the elbow flexed at an angle less than 110° After a pause of 30 sec the sustained contraction was repeated and in the same way maintained as long as possible It was repeated after pauses of 30 sec duration as long as the subject was able to keep the weight lifted for at least 15 sec

A typical work of this type is shown by Fig 1 The mean duration of the first contraction was 136 sec (range 94—167) and with 30 sec pauses on an average 15 contractions were performed The average total time of contractions was 544 sec Every other subject used the right arm and every other subject the left arm

Rhythmic isometric muscle contractions were performed one day after the maximal sustained isometric contraction with the opposite arm The muscle contractions were made in the same way as in the sustained contractions only that the 10 kg weight was kept lifted for a period of 2 sec with 2 sec pauses During the pauses the arm rested on a stand immediately below elbow height The weight was placed so that it could be lifted with a minimum of arm motion The contractions were continued until the total time during which the weight had been lifted was equal to the total time of contraction during the sustained static work of the same individual The total duration of contractions and pauses of the experiments with rhythmic contractions was almost identical or in some cases a few minutes shorter than that of the experiments with sustained contractions

TABLE I Changes of the EMG of arm brachial b ceps and leg quadr ceps muscles af er arm and leg exerc se respectively on a bicycle ergometer

		Leg exerc se quadricep s $\bar{x} \pm s\bar{x}$ n – 6	Arm exerc se brach al b ceps $\bar{x} \pm s\bar{x}$ n = 4	Brach al b ceps + quadn ceps $\bar{x} + s\bar{x}$ n = 10
Percentage frequency of tetraphas c action potent als	$(\bar{x}_1)$	9 6 ± 1 07	9 1 ± 1 9?	9 4 ± 0 91
	$(\bar{x}_2)$	12 2 ± 1 27	11 4 + 1 41	11 8 ± 0 90
	$(\bar{x}_2 - \bar{x}_1)$	+ 2 6¹	+ 2 3	+ ?5
Percentage frequency of polyphas c action potent als	(x_1)	4 8 ± 0 5?	4 4 ± 0 51	4 7 ± 0 36
	(x_2)	7 4 + 1 07	7 1 0 27	7 3 – 0 63
	$(x_2 \; x)$	+ 2 6	+ 2 7³	+ ?6
Mean duration of all act on potent als m.sec	(x)	7 6 ± 0 27	6 8 0 24	7 2 + 0 ??
	x_2	7 2 + 0 36	5 7 + 0 10	6 6 + 0 2?
	$(x_2 - x$	0 4	1 1	0 65
Pulse rate at the end of the highest w ork load b ats/m in		19? + 4 1	180 2 3	
Lactate concentrat on at the e d of the h ghest work load or 3 after the exercise mM l		7 8 + 0 21	6 8 0 83	

x = arithmet c mean x before work x after work
sx = standard error of the mea
¹ – denotes P 0 05 ² denotes P 0 01 ³ deno es P 0 001 where
P is the probab l y that the d fference be w een means is ca se l b ch ance
n the n mber of s bjects exam ned

Techn que fo reco d g and e aluat on of EMG The electro ny grapl a t on poten als were recorded during a sl gl t voluntary contract on bef re as well s af er exer se Iro n 30–40 pos tions as evenly d stributed as poss ble w h n the ent re us le un ler exat nat n The needle electr xdes were nserted perpend cular t the long ax s f t e us le

In exper nents w l arm exercise the l ng l ea l of the brach al l ps u le and n experi ments w th leg exer ise the quadriceps n uscle m n vast lateral s e ed al s were exam ed The percen age frequency f act on poten als w th f ur and f e r n te pl ases r ra f polypl as c action pote t als as determ ned The nu ber f defle s f a act o i p en al was evalua ed acc rd ng to Buchtal *et al* 19?? a The tal lura an l in exper men s s h sus a ned s n e r c c n ract ns the p rk t peak am pl tude f the s e a t n p en als were also measured Because f a skew d str bu n f t e a pl tu les he n ed a ampl ude of an ind v dual was determ ned f r eacl exper r e tal s tuat O l a n poten ials which returned w th dent cal appearance n a less 3 occas ors were lu led The calculat ns were based on at least 100 ac n poten als f rach n l dual a ea l exper ental cond ion

The results of the easurem ents bef re an l after exerc se were btai ned f om the same muscle except n the case f he exper n en s w l s n etr c ntract ons In these exper men s the values bef re exerc se are he n ean f measuren ents fr m t e b cep ?f the r ght and l f arm

Apparatus The muscle act on p ent als were rec r led w th the a l of c ncer t c needle electrodes (DISA 13 KO 3 They l ad a l a eter f 0 (mn an l he area of the inner pla

TABLE II EMG changes in the biceps muscle after sustained (isometric) contractions and after rhythmic (isometric) contractions with the elbow flexors The sum of the periods of contraction in either type of work was 545 (380—717) seconds, mean (range), and the load was 10 kg

		1, sustained contractions $\bar{x} \pm s_{\bar{x}}$ n = 10	2, rhytmic contractions $\bar{x} \pm s_{\bar{x}}$ n = 10	Difference 1—2 $\bar{x}$
Percentage frequency of	($\bar{x}_1$)	9.6±0.59	9.6±0.59	
tetraphasic action potentials	($\bar{x}_2$)	14.8±1.05	10.1±0.53	
	($\bar{x}_2-\bar{x}_1$)	+5.2[3]	+0.5	+4.7[3]
Percentage frequency of	($\bar{x}_1$)	4.1±0.49	4.1±0.49	
polyphasic action potentials	($\bar{x}_2$)	11.9±1.30	5.3±0.47	
	($\bar{x}_2-\bar{x}_1$)	+7.8[3]	+1.2[1]	+6.6[3]
Mean duration of all action	($\bar{x}_1$)	7.3±0.20	7.3±0.20	
potentials, m sec	($\bar{x}_2$)	6.1±0.15	6.9±0.22	
	($\bar{x}_2-\bar{x}_1$)	−1.2[3]	−0.4[2]	+0.7[2]
Lactate concentration 3 min after exercise, mM		1.6±0.23	1.1±0.16	+0.5[1]

Symbols as in Table I

num lead was 0 07 sq mm A three channel DISA electromyograph (13 A 69) was used The [illegible]

by Strom (1949)

Results

Rhythmic work on the bicycle ergometer

The results are given in Table I Before the exercise the percentage frequency of tetra- and polyphasic action potentials was equal in biceps and vastii quadriceps muscles After exercise with arms as well as with legs it had increased equally as much as in the biceps and the vastii muscles respectively The increase was statistically significant for the polyphasic action potentials The duration of the action potentials tended to decrease after the exercise The exercise was maximal or close to maximal as shown by the high pulse rate and lactate concentration

Isometric sustained and rhythmic contractions Before exercise the frequency of tetra- and polyphasic action potentials in the biceps muscle, of those subjects who performed isometric muscle contractions was almost identical with that of the subjects who cycled on the ergometer After the sustained isometric contractions with the elbow flexors, the percentage frequency of tetra and polyphasic action potentials of the biceps

TABLE III Duration and amplitude of various types of action potentials before and after sustained (isometric) contractions of long duration

	Mean duration, m sec ($n=10$)			Median amplitude, μV ($n=10$)		
	Before	After	Difference, before-after	Before	After	Difference, before after
1—3 phasic	7.1	5.6	1.5[1]	188	197	− 9
Tetra-phasic	8.5	7.0	1.5[2]	220	267	−43
Polyphasic	9.0	7.9	1.1[2]	287	283	+ 4
All action potentials	7.3	6.1	1.2[2]	196	212	−17

Symbols as in Table I

TABLE IV Difference in duration and amplitude between 1—3-phasic and tetra- or poly-phasic action potentials before and after work

	Difference in duration m sec ($n=10$)		Difference in amplitude, μV ($n=10$)	
	Before	After	Before	After
Tetraphasic — 1—3 phasic	1.4[2]	1.4[2]	38[2]	72
Polyphasic — 1—3 phasic	1.9[2]	2.3[2]	99[1]	87[1]

Symbols as in Table I

muscle increased more than after the rhythmic isometric contractions (Table II). The effective total duration of the rhythmic contractions was equal to that of the sustained contractions but there was only a slight increase in the frequency of polyphasic action potentials after the rhythmic contractions as compared with the frequency before the exercise.

The duration of the action potentials decreased after the isometric contractions and more after sustained then after rhythmic contractions (Table II). This change is more obvious if the action potentials are divided into three groups (1) mono-, di- and triphasic, 2) tetraphasic and 3) polyphasic (Table III and IV). The tetra- and polyphasic action potentials have a longer duration than the mono-, di- and triphasic ones (Table IV), and they increase in number after exercise (Table II). They therefore tend to counteract a decrease in the mean of the duration of all action potentials.

The amplitude of the action potentials was measured only before and after the sustained contractions. There was no statistically significant change in the amplitude of either type of the action potentials (see Table III). The tetra- and polyphasic action potentials had a higher amplitude than the 1—3-phasic potentials before, as well as after exercise (Table IV).

The lactate concentration (Table II) was somewhat higher after the sustained contractions than after rhythmic contractions ($p < 0.05$)

Reversibility of the EMG changes In 4 subjects the EMG of the biceps muscle was also examined one day after the maximally sustained isometric contraction The frequency of the tetra- and polyphasic action potentials, which was high immediately after the exercise, had on the following day reached the pre-exercise level

Discussion

The results show that after strenuous muscular exercise, particularly after long sustained contractions, there appeared in healthy subjects an increased percentage of tetra- and polyphasic action potentials in the EMG The changes were reversible At the same time, the mean duration of the action potentials decreased

The observed EMG changes may be caused by several factors, presumably related to local changes in the working muscles, for instance accumulation of metabolites or changes in muscle temperature during and after the muscle contractions

Buchtal *et al* (1954 b) observed an increase of polyphasic action potentials at a low muscle temperature Other changes in temperature might therefore influence the EMG In some measurements, made under similar conditions as the present experiments, temperature changes of the working muscles were found to be greatest during and after the exercise on the bicycle ergometer After this type of exercise the EMG changes were small During the sustained and rhythmic contractions the rise in temperature was small and at the time of recording the EMG the muscle temperature had regained the pre exercise level (Bjerle 1964) Changes in temperature, therefore, do not seem to have caused the observed EMG changes

It seems more likely that excessive accumulation of metabolites contributed to the EMG changes They occurred most marked after the sustained contraction when the accumulation of metabolites probably was the greatest Thus previous examinations indicate that the muscle blood flow in relation to metabolism is smaller in sustained

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after exercise was comparatively small and of short duration (Kramer and Quensel 1937, Barcroft and Swan 1953 Shepard 1963 and others)

Lindhard (1920) studied static effort in which the subject hung by the hands from a beam with bent arms He found that the greater part of the increase in oxygen uptake and in the calculated cardiac output occurred after the exercise had ended and he concluded that in strong contractions the blood flow to the muscles was occluded by mechanical compression Similar observations were described by Asmussen and Hansen (1938) In the soleus muscle of man the blood flow was observed to be arrested if the force of the sustained contraction was more than about 20 % of the maximal voluntary contraction (Barcroft and Millen 1939) In the forearm of man the blood flow seems to decrease during hand grip contractions stronger than about 70 % of the maximal voluntary contraction (Humphrey and Lind 1963) These strong contractions results in exhaustion and become intolerable within a few min

In experiments in which the sustained isometric contractions were moderate or not well defined with regard to their strenght it has been observed that the blood flow through the contracted muscle was increased in comparison to that of the uncontracted

muscle. After the contraction, however, there was a reactive hyperaemia (Kramer and Quensel 1937, Barcroft and Millen 1939, Lind *et al.* 1964), indicating that in these contractions there was also a relative obstruction to the blood flow.

The load that was applied in the sustained contractions of the present experiments corresponded to about 1/3 of the maximal voluntary contraction (Asmussen and Heelbol Nielsen *et al.* 1961). The contractions were tolerable only for a few (2—3) min (Fig. 1). It therefore seems likely that the circulation was arrested, or at least relatively obstructed, during the sustained contraction giving the prerequisites for an accumulation of metabolites. The concentration of lactate was also somewhat higher in the blood after the sustained contractions than after the rhythmic but in other respects comparable contractions.

The increase in lactate concentration of finger blood during exercise reflects the total production of lactate. The lactate production resulting from the two types of work with small but equally large muscle groups was quite small in comparison with that obtained with cycling, when the large muscle groups of the legs were used. However, it seemed to be larger with sustained than rythmic contractions. Larger differences between the various types of work might have been obtained if lactate concentration of venous blood from the working muscles had been examined.

The following facts have some bearing on changes in muscles that may be caused by marked accumulation of metabolites and/or that might correspond to the EMG changes observed. a) Anatomical changes with cell degeneration have been reported after muscle work combined with insufficient circulation and/or accumulation of metabolites in horses (azoturia or Kreutzlahme, see Carlström 1931) and in the tibialis anterior syndrome in young men (Severin 1943, Horn 1945, Carter *et al.* 1949 and others). b) Further an increased number of polyphasic action potentials and a decrease of action potential duration is characteristic of various myopathies with anatomical changes in the muscles (Kugelberg 1947). c) Finally, similar EMG changes were marked after slight exercise in patients with a hereditary myopathy with paroxysmal myoglobinuria (Larsson *et al.* 1964). The changes were reversible and were most likely connected with a disturbed muscle metabolism during exercise, resulting in an abnormally high concentration of lactate and pyruvate in muscle blood in relation to the work load. Although no anatomical changes were observed in the muscles under these conditions, it seems likely that the functional changes that were reflected in the increased number of polyphasic action potentials etc. may precede the more advanced changes with muscle cell degeneration which were observed in the acute state of the disease. Such muscle degeneration was often provoked by prolonged and strenuous exercise.

Other factors than those discussed may cause the observed EMG changes. So far it may be concluded, however, that reversible EMG changes of this type seem to occur in pathological states as well as in normal muscles after exercise.

The nature of the changes which caused the observed EMG pattern is not known. According to Kugelberg (1947) the polyphasic action potentials and the shortened duration of action potentials that appear in various myopathies may be due to a successive elimination of muscle fibres. Such a mechanism, although reversible, may also operate after exercise in normal subjects. If so, it is difficult to understand why the mean duration of the tetra- and polyphasic action potentials is longer and the median value of the amplitudes higher than those of mono-, di- and triphasic action potentials before, as well as after the exercise. See Table III and IV.

Other explanations to the increased number of polyphasic action potentials might be a change in the conduction velocity of the muscle fibres or a change in the transmission in the myoneuronal synapsis which both may be influenced by metabolic changes in the muscles

Buchtal *et al* (1954 b) discuss the possibility of an increased synchronization between motor units as a cause of the increased number of polyphasic action potentials after exercise If this was the cause, it is difficult to understand why the increased number of polyphasic action potentials then appears particularly after the long sustained contractions or why the mean duration of tetra and polyphasic action potentials decrease

It is obvious that several explanations of the observed EMG changes are possible The knowledge that a reversible increase of polyphasic action potentials and a decreased duration of the action potentials can be induced especially by sustained muscular contractions facilitates further studies Such studies may also contribute to a better understanding of the cause of the EMG changes in certain myopathies A knowledge of the EMG changes caused by exercise may also be valuable for the study of conditions with overstrained muscles This occurs for instance in occupations with unsuitable working positions which require sustained muscular contractions of long duration in certain muscle groups

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Effects of Various Anesthetics and of Surgical Preparation on Acid-Base Balance in Cats

By

Erik Berglund[1], Olle Nylen and Ingemar Wallentin

Received 10 March 1965

Abstract

In physiological laboratories extensive studies are performed after administration of anesthesia and surgical preparations In most studies acid base and ventilatory data are not presented Bygdeman (1963) has shown deleterious effects of respiratory acidosis on vascular reactivity The acid base state in long duration physiological experiments on cats has not been investigated

The present study will show the effect of some commonly used physiological' anesthetics on the acid base balance in cats which have not been exposed to surgical preparation Further, the effect of added extensive surgical preparation was studied The study was prompted by some preliminary experiments in dogs (Norrsell and Berglund, personal communication) showing metabolic acidosis after ether and surgical preparation and after nembutal and surgical preparation but not after nembutal alone

Procedure

Experiments were performed on cats weighing 1 8—4 4 kg Anesthesia was induced with diethylether As soon as the cat was asleep a cannula was inserted into the trachea to assure free air passage and another cannula was inserted into a femoral vein The anesthetic to be studied was then immediately injected Most of the cats were given heparin i v (5 mg kg b w)

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TABLE I Values of pH at P_aCO_2 28 after various anesthetics Hours after start of drug ad

		1 st	2 nd
Nembutal	Range	7 32–7 50	7 32–7 46
	Mean	7 40	7 38
n=5	Number of cats	n=4	n=4
Chloralose alone	Range	7 31–7 43	7 32–7 48
	Mean	7 37	7 38
n=12	Number of cats	n=6	n 11
Urethane alone	Range	7 32–7 42	7 27–7 37
	Mean	7 35	7 32
n=4	Number of cats	n–3	n 3
Chloralose urethane	Range	7 21–7 35	7 27–7 35
	Mean	7 31	7 31
n 10	Number of cats	n=9	n=7
Urethane added after chloralose	Range	7 37 7 39	7 32–7 35
	Mean	7 38	7 34
n=5	Number of cats	n 3	n=4

in parallel and the sample time was therefore not absolutely regular

In 12 cats surgical preparation was also performed this included extirpation of the it mach and most of the intestines and in most cases also the pancreas These studies were done only in cats anesthetized with nembutal or chloralose

Anesthetics

The doses of the individual anesthetics were those commonly used in this laboratory Sodium pentobarbital Nembutal® Abbot 30 mg/kg Chloralose 50 mg kg Chloralose + urethane 50 mg kg 100 mg kg Urethane 1 g kg In a few experiments Chloralose was given as described above and urethane in a dose of 100 mg kg was added a few hours later

Analyses of pH and CO_2 tension

Arterial blood samples of 1 2 ml were taken in a 2 ml syringe In those experiments were no heparin had been given [illegible] The pH was [illegible] Aliquots [illegible] CO_2 [illegible] gases were analysed with the Scholander technique (Scholander 1947 All pH measurements were made at 38 C

[illegible] 19[illegible] using the [illegible] sample [illegible] tive CO_2 [illegible] the actual

ministration

3 rd	4 th	5 th	6 th—7 th
7 42—7 49	7 27—7 48	7 32	7 27—7 44
7 46	7 36	7 32	7 36
n=3	n=4	n=2	n=4
7 31—7 43	7 34—7 42	7 37—7 40	7 38—7 43
7 38	7 39	7 39	7 41
n=9	n=4	n=2	n=2
7 30—7 40	7 29—7 37	7 37	7 28
7 35	7 34	7 37	7 28
n=4	n=4	n=1	n=1
7 19—7 35	7 17—7 35	7 20—7 29	7 24—7 45
7 28	7 28	7 25	7 32
n=8	n=9	n=2	n=5
7 33—7 37	7 33		
7 35	7 33		
n=3	n=1		

Results

The data for each anesthetic drug or combination are presented in Table I

A few samples were discarded because of technical accidents, occurring especially during the equilibration procedure Only experiments with at least two samples are included

For estimation of metabolic" or non-respiratory changes in acid base balance, the pH at $P_{a_{CO_2}}$ = 28 mm Hg is given The $P_{a_{CO_2}}$ value of 28 was chosen with reference to the finding reported by Fink and Schoolman (1963) that the normal $P_{a_{CO_2}}$ value in unanesthetized cats is 28 ± 4 mm Hg The average pH value in their study was 7 38 ± 0 06

During the first 3 hrs after *nembutal* administration the $P_{a_{CO_2}}$ 28 values (Table I) reveal no metabolic acidosis Thus, any metabolic acidosis induced by the brief ether anesthesia, is abolished by this time After the third hour two of the 5 cats showed slight metabolic acidosis

After *chloralose alone* none of the twelve cats developed metabolic acidosis during or after the first 3 hrs

In contrast, 6 of the 10 cats with *chloralose + urethane* showed a metabolic acidosis usually from the first sample, it remained rather constant except in one cat Further, one cat showed borderline values

In order to further elucidate the difference between chloralose alone and chloralose together with urethane, separate studies were undertaken Four cats were anesthetized with *urethane alone* (Table I) one cat developed metabolic acidosis In 5 other cats *urethane* was added after 1 1 2—3 hrs of chloralose anesthesia The values after addition

TABLE II Values of pH at P_aCO_2 28 after various anesthetics and surgical preparation Hours

		1st	2nd	3rd
Nembutal	Range	7 28 – 7 41	7 29 – 7 39	7 32 – 7 41
	Mean	7 36	7 34	7 36
n = 6	Number of cats	n – 12	n – 9	n = 6
Chloralose	Range	7 20 – 7 40	7 33 – 7 37	7.28 – 7 33
	Mean	7 32	7 35	7 31
n = 6	Number of cats	n = 10	n = 3	n = 4

of urethane are given in Table I In none of the 5 cats did any metabolic acidosis develop

In 9 of 31 cats the arterial CO_2 tension exceeded 32 mm Hg at some time, but in no cat was there any marked respiratory depression In 7 cats the arterial P_{CO_2} was clearly below 24 mm Hg already early in the study No group showed any consistent tendency toward hypo- or hyperventilation Small changes were seen in many cats during the course of anesthesia

In the surgically prepared cats anesthetized with nembutal (6 cats) or chloralose (6 cats) there was after 3 1/2 hours a progressive metabolic acidosis (Table II) In the nembutal cats there was a moderate alveolar hyperventilation

Discussion

Nembutal chloralose and urethane when given separately did not produce any marked disturbances in acid base balance

On the other hand it was surprising to observe that administration of chloralose and urethane together was accompanied by metabolic acidosis in more than half of the experiments The reason for this is obscure Addition of urethane after 1 1/2 to 3 hrs chloralose anesthesia did not give rise to metabolic acidosis In our laboratory chloralose urethane has until recently been a commonly used anesthetic The present results indicate that it may be more advantageous to use chloralose alone or with later addition of urethane On the other hand anesthesia with urethane alone was difficult to maintain and was accompanied by extreme tachypnoea without alveolar hyperventilation

after start of drug administration

4 th	5 th	6 th	7 th
7 24—7 37	7 24—7 33	7 12 7 34	7 22
7 33	7 29	7 25	7 22
n=8	n 5	n=5	n 1
7 30—7 44	7 25—7 33	7 17—7 30	7 13—7 25
7 35	7 29	7 24	7 19
n=5	n=3	n 4	n=2

The finding that acidosis is found after *injection of a chloralose-urethane mixture* but not when the two are given separately a few hours apart is puzzling It is possible that one of the drugs potentiates the other as regards metabolic processes, and that this potentiation can not occur once one of the drugs has been absorbed to the tissue The solution of a mixture of chloralose and urethane was no more acid than the solutions of chloralose and urethane alone

Although nembutal and chloralose *per se* do not produce metabolic acidosis, this complication is probably a common one in many "physiological" *experiments* The data from those nembutal or chloralose cats who underwent extensive surgical prepara tion demonstrate one important cause of metabolic acidosis

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Catecholamine Depletion and Uptake in Adrenergic Nerve Vesicles and in Rabbit Organs after Decaborane

By

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Received 11 March 1965

Abstract

Euler, U. S. v. and F. Lishajko. *Catecholamine depletion and uptake in adrenergic nerve vesicles and in rabbit organs after decaborane.* Acta physiol. scand. 1965. 65. 324—330. — Decaborane ($B_{10}H_{14}$) increases the release rate of noradrenaline (NA) from isolated nerve vesicles in concentrations of 10 [illegible] M [illegible] in nerve vesicles incubated in the presence of NA [illegible] M which also inhibits the ATP-dependent NA [illegible] gradually decreases the catecholamine stores in various organs of the rabbit. Between 24—48 hours after the decaborane injection the NA content in the heart is about 10—20 per cent of the normal value. Injection of NA 0.3—0.4 mg/kg i.m. increases the NA content in the heart to near normal in 1—2 hrs, whereafter it falls to the previous low value in some 20 hrs.

The NA taken up by the rabbit heart after depletion with decaborane is distributed between the high speed sediment and the supernatant in the same proportions as found in the normal heart suggesting a dynamic equilibrium between the vesicles and the non vesicular pool.

It has recently been shown that a boron hydride, decaborane ($B_{10}H_{14}$), lowers the noradrenaline (NA) content of the rat brain (Merritt, Schultz and Wykes 1964). The present paper deals with some effects of decaborane on isolated bovine nerve vesicles and on the NA content of various organs of the rabbit, including different fractions of the homogenized rabbit heart. In addition the uptake of NA and A in several organs and in heart fractions after administration of the amines to decaborane treated animals has been studied.

Methods

Isolated nerve vesicles

[illegible]

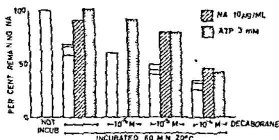


Fig. 1. Effect of decaborane on the release of NA from isolated splenic nerve vesicles on incubation 0.13 M potassium phosphate, pH 7.5 at 20° for 60 min. Ordinate: NA remaining in sediment after incubation. Empty bars: Decaborane alone added as indicated on abscissa. Hatched bars: Decaborane in the presence of NA 10 μg/ml. Dotted bars: Decaborane in the presence of ATP 3 mM. The retarding effect on NA release of added NA or ATP (column 3 and 4) is almost annulled by decaborane 10^{-4} M (last 2 columns)

ments. At the end of the incubation period the suspension was centrifuged for 30 min at 50 000 × g

Rabbit organs

with acetic acid and estimated fluorimetrically.

Decaborane in a concentration of 10^{-3} M added to the extract did not influence the recovery of NA in the extract when subjected to passage over alumina and elution in the same way as the extracts.

Noradrenaline and adrenaline were injected i.m. in doses of 0.1–0.4 mg/kg or i.v. 0.05 mg/kg

Heart fractions

Results

Isolated nerve vesicles

The NA release rate on incubation of isolated nerve vesicles in 0.13 M potassium phosphate at pH 7.5 has been determined previously at different temperatures (Euler and Lishajko 1963). In the present experiments the vesicle suspension was incubated for 60 min at 20° or 10 min at 37° in the presence of decaborane in concentrations of 10^{-6} to 10^{-4} M. As seen in Fig. 1 the release rate was not affected by decaborane 10^{-6} M but increased at 10^{-5} and 10^{-4} M. At 10^{-3} M concentration (not shown in figure) the release rate was greatly increased, the half life at 37° being decreased from about 10 min to 2—3 min. Since the NA release rate is markedly increased with decaborane 10^{-4}

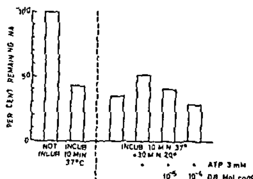


Fig 2

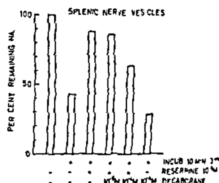


Fig 3

Fig 2 Effect of decaborane on the uptake of NA into isolated splenic nerve vesicles in the presence of ATP 3 mM after partial depletion NA concentration 6 nmol/ml Incubation medium as in Fig 1 Incubation times and temperature as indicated

Fig 3 Effect of decaborane on the release of NA from isolated splenic nerve vesicles in the presence of reserpine 10^{-6} M Incubation medium as in Fig 1

M and higher concentrations even at + 2°, it appears that the effect in these concentrations is due, at least partly, to some damage of the storage vesicles

When incubated in the presence of 10 μg/ml NA the vesicles retain their NA content as previously described, owing to simultaneous uptake (Euler, Stjarne and Lishajko 1963) Decaborane at 10^{-6} M and 10^{-5} M had a relatively small effect upon this retention but almost eliminated it at 10^{-4} M (Fig 1) A similar result was obtained with decaborane and ATP, suggesting that decaborane 10^{-4} M blocks the uptake of NA in the vesicles, otherwise occurring in the presence of NA and of ATP (Fig 1)

The enhancing effect of ATP on the NA uptake after previous partial depletion is inhibited by decaborane 10^{-5} M and totally blocked at 10^{-4} M (Fig 2)

The effect of decaborane was also studied on the inhibition of release caused by reserpine on vesicles While 10^{-5} M decaborane did not influence the retarded release rate caused by reserpine 10^{-6} M a higher concentration of decaborane, 10^{-4} M considerably increased the release rate even in vesicles exposed to reserpine However, the reserpine inhibition of release was only partially counteracted by this concentration of decaborane (Fig 3)

Decaborane thus belongs to the group of substances which in all concentrations enhances the release of NA from the vesicles

Action of decaborane on the NA content of rabbit organs

The result of i p injections of decaborane on the NA content of rabbit organs is shown in Table I and Fig 4

From Table I and Fig 4 it is seen that the NA content falls relatively slowly and remains low from 24 to 48 hrs after a dose of 4 mg/kg of decaborane In the brain, spleen and adrenals the depletion starts more slowly than in the heart and the kidneys At 72 hrs the NA content of the organs is again increasing A comparison with the data of Merritt *et al* (1964 shows that the depletion in the rat brain after a dose of 15 mg kg occurs considerably faster than in the rabbit's brain after 4 mg kg As seen in Table I

TABLE I NA content ($\mu g/g$) in rabbit organs various times after intraperitoneal injections of decaborane 4 mg/kg Adrenal values refer to adrenaline in $\mu g/kg$ b w Mean ± SEM

Time after decaborane hrs	Heart	Brain	Spleen	Kidneys	Adrenals	n
Controls	1 72 ± 0 18	0 17 ± 0 01	0 37 ± 0 13	0 14 ± 0 01	71 ± 13	(4)
2—6	1 01 ± 0 15	0 16 ± 0 01	0 33 ± 0 08	0 041 ± 0 008	51 ± 5 2	(8)
24	0 17 ± 0 033	0 054 ± 0 008	0 053 ± 0 023	0 017 ± 0 004	29 ± 6 2	(4)
48	0 19	0 045	0 074	0 024	25	(2)
72	0 56 ± 0 17	0 094 ± 0 006	0 090 ± 0 036	0 14 ± 0 037	65 ± 12	(3)

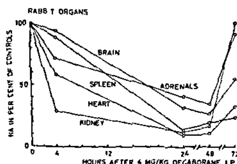


Fig 4

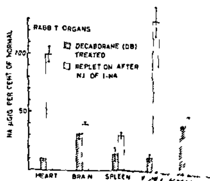


Fig 5

Fig 4 NA content in rabbit organs in per cent of normal various times after intraperitoneal injection of 4 mg kg decaborane

Fig 5 NA content in rabbit organs in per cent of normal after depletion with decaborane 24 hrs previously (hatched bars) and 1—2 hrs after 0 3—0 4 mg kg NA intramuscularly (empty bars)

and Fig 4 the depletion is considerably less marked in the brain and the adrenals than in the other organs studied

[illegible]

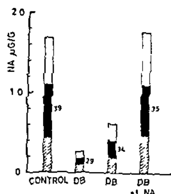


Fig. 6. NA content in fractions of homogenized rabbit heart in controls, in 2 groups of decaborane treated animals and in animals having received l-NA after depletion caused by decaborane. Hatched bars: Low speed sediment; black bars: High speed sediment; empty bars: high speed supernatant.

study in detail the relationships between doses and the depleting effect. For the following experiments a standard dose of 4 mg/kg i. p. was used in order to cause 80—90 per cent depletion of the NA content of the heart. The animals were used for uptake experiments 24—48 hrs after the decaborane injection.

Uptake of catecholamines after depletion with decaborane

Doses of 0.3—0.4 mg/kg l-NA i. m. in animals previously depleted with decaborane refilled the NA stores in the heart in 1—2 hrs (Fig. 5). Smaller doses or shorter intervals resulted in smaller uptake. Doses of 0.05 mg/kg i. v. caused an uptake to near normal values in 5 min to 2 hrs.

The NA content in the depleted kidneys was increased to normal or even higher than normal in 1—2 hrs after doses of 0.3—0.4 mg/kg i. m. or in 5 min following an i. v. dose of 0.05 mg/kg. In the spleen the uptake was significant but of a low order, whereas in the brain and the adrenals no significant uptake was observed.

Organs analyzed about 20 hrs after the NA injection in decaborane depleted animals did not differ significantly in their NA content from those obtained after decaborane alone, indicating that the amount taken up has left the organs within this period.

In NA-depleted animals the uptake of l-A was similar to that of l-NA.

NA uptake in heart fractions

Administration of large amounts of NA to a normal animal may temporarily increase the NA content of various organs. As shown by Wegmann and Kako (1961) and Campos and Shideman (1962) this uptake is chiefly in the "soluble" fraction of the heart and may therefore be regarded as largely unspecific. In order to ascertain whether the total uptake of NA in our experiments was a true uptake, i. e. into the specific vesicular stores, hearts were homogenized, fractionated by differential centrifugation and the amounts determined in the various fractions. As seen in Fig. 6 the amount of NA in the high speed sediment increased roughly proportionally to the total amount after administration of the amine. The amount of NA in the high speed sediment constituted about 50 per cent of the total NA content in the heart, less the coarse fraction, irrespective of the total amount. The relatively fixed proportion suggests an equilibrium between the NA

in the high speed sediment containing the storage particles and in the supernatant (Euler and Lishajko 1965a) The uptake of relatively large amounts of NA into the high speed sediment occurs within a few minutes after i v injection

Discussion

presence of decaborane 10^{-2} M The depletion occurs at different rates in different organs, being relatively slow in the brain and the adrenals, and more rapid in the heart and the kidneys The effect is, on the other hand, of a fairly long duration which facilitates uptake studies Thus low NA values were consistently found over the period between 24 and 48 hrs after injection of decaborane in a dose of 4 mg/kg i p

speed sediment and the supernatant, indicating a dynamic equilibrium between these stores, as suggested for the adrenal medullary cells by Hillarp (1960) and also for the nerves (Euler and Lishajko 1962, Stjarne 1964, Michaelson *et al* 1964) In some of these studies, as in the present one, the rapid uptake of NA into the stores was noted

Preliminary experiments have shown that NA and A are taken up to about the same extent after decaborane depletion This question and the relative uptake of the d and l isomers will be discussed in a subsequent paper (Euler and Lishajko 1965b)

With the technique used no definite amine uptake in the brain was observed, which is consistent with the known fact that the amines enter the brain only in very limited amounts Neither was a reloading noticeable in the adrenals probably owing to the fact that a small uptake will hardly affect the content in the adrenal medulla whereas the same amount per weight unit taken up by the heart for example, will increase its content considerably Thus an uptake of 1 μg/g organ will be easily detected in the heart, while the corresponding total uptake of less than 0 5 μg in the adrenals will pass unnoticed when nonlabeled compounds are used

The proportions of NA in the different heart fractions were found to be approximately constant at all levels of NA in the heart after depletion with decaborane as well as after administration of l NA i m However, if NA was given i v the relative amount of NA found in the high speed supernatant was moderately increased during the first 5—10 min after the injection with the doses used Several explanations could be offered for this lag in reaching the final proportions but the most likely one appears to be that part of the circulating NA is present in the extracellular fluid or is attached to some structures which hold the amine only for a short time After large doses the proportion of NA in the supernatant may temporarily be very high (Wegmann and Kako 1961

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Thyroidectomy of the Goat

By

LARS EKMAN

Received 23 March 1965

Abstract

[illegible]

no clinical manifestations of hypothyroidism were detected during the seven month period the animals were under observation

In connection with studies of the interaction between nervous and hormonal thermoregulatory mechanisms in the goat (cf Andersson *et al* 1963) it became desirable to use also thyroidectomized animals It was however, soon observed that simple surgical thyroidectomy in the goats did not result in an athyroid state The method used to obtain such a state and some observations made during the development of athyroidism will be reported in the present paper

Methods

Animals 6 adult goats were used 4 of the goats (no I—IV) were non pregnant and had silver thermodes for local brain cooling implanted medially in the rostral hypothalamus (cf Andersson *et al* 1963) The remaining two goats (no V and VI) were intact and in early pregnancy at the time of surgical thyroidectomy

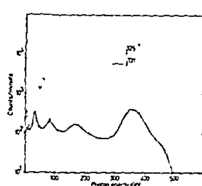


Fig 1

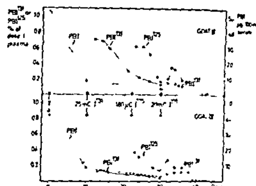


Fig 2

Fig 1 Gamma ray spectrograms of samples containing 0 1 uCi I^{125} and 0 028 uCi I^{131} respectively

Fig 2 The effects of treatment doses of I^{131} on PBI PBI^{131} and PBI^{125} goats III and IV

Isotopes Carrier free I^{131} (Na I^{131}) delivered by "Institutt for Atomenergi", Kjeller, Norway was used I^{125} as iodide in M/50 aqueous thiosulphate was obtained from "The Radiochemical Centre", Amersham England According to the manufacturer, the I^{125} preparation contained less than 0 3 per cent I^{126} The gamma energy peak at 382 KeV of I^{126} will interfere when measuring the 364 KeV gamma emission of I^{131} By storing the I^{125} preparation for about 8 weeks most of the I^{126} decayed out

Surgical thyroidectomy and radioisotope scanning Prior to thyroidectomy the goats were given a tracer dose of 40 to 60 uCi I^{131} Maximum radioiodine uptake and plasma PBI^{131} were measured in the same way as previously described (Andersson *et al* 1963) Surgical thyroidectomy was then accomplished under nembutal anaestesia To detect ectopic thyroid tissues following the surgical thyroidectomy a scintillation detector with a 2 2 NaI (Tl) crystal and furnished with a lead collimator FH Bl 509 (Frieseke and Hoepfner) was used The collimator inset was cylindrical and had an orifice 33 mm in diameter and a length of 89 mm The orifice of the collimator inset was palced against the skin Measurements were made stepwise along the whole ventral neck region and at a few points of the ventral border of the chest These scanning measurements were also performed 7 to 14 days after the later treatment of the animals with radioiodine

Radiothyroidectomy To destroy all accessory thyroid tissue remaining after surgical thyroidectomy the animals were given large doses of I^{131} It was desirable to get a complete destruction of thyroid tissue without producing any apparent general radiation effect The hematological [illegible]

[illegible] gamma ray spectrograms obtained after measurement of two samples containing 0 1 uCi I^{125} and 0 028 uCi I^{131} respectively The 364 KeV gamma emission of I^{131} can be measured without interference by I^{125} A correction however must be made for the I^{131} contribution to the I^{125} count at 27 4 KeV and 35 4 KeV The energy interval 10 90 KeV was chosen for I^{125} + I^{131} and 314—420 for I^{131} determination Standards were prepared for the appropriate administered doses

TABLE I I^{131} treatment scheme of the surgically thyroidectomized goats and the results of radioisotope scanning on the ventral surface of the goats' neck and chest. The time interval between each treatment dose of I^{131} varied between 20 and 35 days

Animal no	1 st dose		2 nd dose		3 rd dose		4 th dose		Localization of residual thyroid tissue
	mCi I^{131}	Scanning	mCi I^{131}	Scanning	mCi I^{131}	Scanning	mCi I^{131}	Scanning	
I	0.75	+	10	+	20	+			In front of the heart
II	20	+	25	−	25	−			In front of the heart
III	25	+	20	+	25	−	25	−	In front of the heart
IV	25	−	20	−	25	−			—
V	25	+	25	+	25	−	25	−	Between the middle and lower third of the neck
VI	25	−	25	−	25	−			—

Biochemical determinations At varying intervals determinations were made of serum protein bound iodine (Barker and Humphrey 1950)[1] and serum calcium (Diel and Ellingboe 1956)
Hematological determinations were made at gradually lengthening intervals of red cell count packed cell volume hemoglobin total and differential cell counts

Results

Before surgical thyroidectomy all goats in this study had a high thyroid uptake of I^{131} A maximum uptake of 46 to 72 per cent of the tracer dose was found 24 to 72 hours after the administration of I^{131} The maximum level of PBI^{131} in the blood plasma before thyroidectomy varied between 0.5 and 3.7 per cent of given dose per liter plasma and was reached on the second to the fourth day after the administration of radioiodine

After surgical thyroidectomy residual tissue could not be detected in any of the goats in the region where the thyroid gland had been situated Ectopic thyroid tissue, however, was found by radioactivity measurements in goats I, II, III and V (Table I) In goat V tissue concentrating radioiodine could be localized to an area at the border between the middle and lower third of the neck, and in the remaining three animals in an area inside the chest just in front of the heart After repeated treatment with I^{131} the ectopic thyroid tissue was destroyed in all goats as judged by scanning

The development of complete thyroidectomy was also studied by measurements of serum PBI and plasma PBI^{131} The results obtained, in two goats, III and IV, can be seen in Fig 2 In goat III ectopic thyroid tissue was detected but not in goat IV The maximum levels of plasma PBI^{131} found before surgical thyroidectomy in these two

[1] Grateful acknowledgement is made to Dr G Widstrom M D Head of the Clinical Laboratory Danderyds Sjukhus Danderyd by whose courtesy the serum PBI determinations were made

goats were 0 6 and 2 7 per cent of the tracer dose per liter plasma respectively After the first post operative treatment dose of I^{131} the plasma PBI^{131} value were 0 7 per cent in goat III and 0 5 per cent of the dose per liter plasma in goat IV (which lacked detectable ectopic thyroid tissue) As in goat III an increase of plasma PBI^{131} was noted in the other animals with detected ectopic thyroids Most pronounced was the rise in goat I in which maximum plasma PBI^{131} before surgical thyroidectomy was 1 3 *per cent of the* dose per liter plasma and after a post operative treatment dose of 10 mCi I^{131} was 16 9 per cent of the latter per liter plasma

By giving a tracer dose of I^{125} (180 uCi) to goats III and IV before administration of the second post-operative treatment dose of I^{131} the effect of I^{131} on PBI^{125} was studied As can be seen in Fig 2 the plasma PBI^{125} rose in both animals showing that I^{125} labelled protein had been released into the blood due to the second treatment dose of I^{131} Also the level of plasma PBI^{131} was estimated immediatly before and after the second treatment dose of I^{131} In contrast to the rise in PBI^{125} there was hardly any increase in PBI^{131}, indicating that hardly any of the I^{131} used in the second treatment dose had been taken up by thyroid tissue

No serum PBI determinations were made on the days soon after the administration of treatment doses of I^{131} but a rise of serum PBI is likely to have occurred However, 2 to 3 weeks after the second treatment dose of 20 to 25 mCi I^{131} the serum PBI was lower than 0 3 ug/100 ml serum in the 6 goats studied

To ensure complete destruction of thyroid tissue the goats were given an additional large dose of I^{131} after the first scanning in which no ectopic thyroid tissue could be detected To control that no thyroid tissue had developed goats II, III and IV were given a tracer dose of I^{131} about three months after the last treatment dose of I^{131} Scanning of the animals at that time did not reveal any residual thyroid tissue nor was any serum detected However in the 3 goats a maximum plasma PBI^{131} ranging between 0 08 and 0 15 per cent of the dose per liter plasma was found None of this PBI^{131} could be extracted with acid butanol from the plasma protein precipitate indicating that the plasma PBI^{131} did not represent thyroid hormones

Other than a considerable increase in body weight and certain changes in temperature regulation (Andersson Ekman and Hökfelt 1965) no change in the general apperarance of the animals was noted Goat IV had a body weight of 32 kg at the time of surgical thyroidectomy and 7 months later the weight was 44 kg The other goats increased their weight by 12 to 25 per cent of the pre experimental weight

No significant hematological changes were seen in any of the goats Serum calcium was also unchanged

Two of the goats no V and VI were in early pregnancy at the time of surgical thyroidectomy 3 months later goat V delivered two kids which were dead at birth Goat VI delivered one kid 3 1 2 months later The kids appeared to be normally developed with the exception that no thyroids could be detected Goat VI had got a tracer dose of I^{131} 6 days before delivery thus making it possible to scan the kid No iodine concentrating tissues could be detected either in the neck region or elsewhere At autopsy nothing abnormal was noticed but inspection of the lungs revealed that they had not respired

In goats V and VI approximately normal amounts of colostrum milk were milked out of the udder on the days of delivery The amounts of milk obtained during the first week after delivery were less than 10 per cent of that obtained from normal goats in the same stage of lactation

Discussion

In the goats two parathyroids are embedded in the thymus, one on either side just above the level of the thyroid cartilage Another pair are intimately bound up in the thyroid proper (cf Ellenberger and Baum 1943) Therefore the thyroid with its associated parathyroids can be removed leaving one pair of parathyroids undamaged In consequence there was no sign of parathyroid hormone deficiency in the goats in the present study

Occurence of ectopic thyroid tissues has been described in other animal species (cf Ellenberger and Baum 1943) The present study shows that it is also common in the goat Therefore if one wishes to completely thyroidectomize a goat the surgical removal of the thyroid gland has to be followed by radioiodine treatment In the present investigation it was desirable to obtain an athyroid state without causing any general radiation effect on the animal A dose of 25 mCi I^{131} repeated at about a month interval was not found to cause any hematological changes or other symtoms of radiation sickness This is not surprising since the integrated whole body dose from 25 mCi I^{131} will not exceed 40 rad, assuming an even distribution in the soft tissues of the goats

In adult sheep a depression in lymphocyte count has been observed following surgical thyroidectomy (Hackett *et al* 1961) or radiothyroidectomy (Bustad *et al* 1957) In the cow, however Garner *et al* (1961) did not see any hematological changes efter radiothyroidectomy There may thus be species differences explaining why no hematological changes was observed in the goats

The sudden rise of PBI^{131} and PBI [illegible] following treatment doses of I^{131} support the view that the iodine concentrating tissue remaining after surgical thyroidectomy really represent thyroid tissue A similar rise has been seen in cattle with intact thyroid gland when the animals received 10 mCi I^{131} daily for 14 days (Garner *et al* 1961) There are indications that some thyroglobulin might be released from the thyroid gland at much lower doses (Owen *et al* 1960 Anbar and Inbar 1963)

[illegible]

reduced metabolic rate in hypothyroidism which causes a reduction in tissue fat catabolism and utilization (cf Macgregor 1964)

The overall clinical changes following surgical thyroidectomy in the sheep are well known (Simpson 1924 Marstom and Pierce 1932 Spielman *et al* 1945) as are the changes following radiothyroidectomy Bustad *et al* 1957) The onset of the symtoms is much more rapid and marked in the young animals than in the adult The lack of drastic clinical manifestations of hypo- or athyroidism in the present goats may therefore be due to the fact that only adult animals were used in the present study

The I^{131} doses to the pregnant goats were large enough to completely destroy the thyroids of the fetuses Other than lacking thyroid glands the offspring appeared macroscopically normally developed The cause of death is therefore not apparent The same observation was made in cattle when the pregnant cow received large doses of I^{131} (Garner *et al* 1961 It would be of interest to study the development of the central nervous system in fetuses and young animals which are athyroid

In previous studies of milk production in hypothyreotic farm animals a reduction has been observed Spielman Petersen and Fitch 1944 Garner *et al* 1961 Further evidence for the need of thyroid hormones for maintenance of normal lactation was provided by

the present study in athyreotic goats in which an almost complete cessation of lactation occurred In laboratory animals normal lactation is said to take place even in the absence of the thyroid (cf Myant 1964) This difference between farm and laboratory animals may be explained by species differences or by an uncomplete thyroidectomy in the studies of laboratory animals

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Adrenomedullary Response to 2-Deoxyglucose in the Hypothyroid, Euthyroid, and Hyperthyroid Rat

By

D G JOHNSON[1]

Received 29 March 1965

Abstract

After the early demonstrations of a relation between the thyroid status of an animal and its catecholamine metabolism (Cyon 1898, Kraus and Friedenthal 1908, Eppinger Falta and Rudinger 1908) several studies were made which attempted to elucidate possible differences in the adrenomedullary response to insulin induced hypoglycemia under various thyroid conditions Hökfelt (1951) found that while adrenal gland levels of adrenaline were regularly higher in the hypothyroid rat and lower in the hyperthyroid rat than in normal rats insulin administration caused less depletion in the hyperthyroid rat than in normal rats while hypothyroid animals exhibited the largest depletion These observations as pointed out by the author, were consistent with either the possibility that hypothyroid rats have a decreased ability to synthesize catechols or there is an increased demand for and secretion of adrenaline " Other studies on the rat

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TABLE I Total body weight and adrenal gland weights of rats on the 22nd day of treatment

	Body weight (g) Mean ± SEM	Adrenal gland weight (mg) Mean ± SEM
Hypothyroid	241±2	30 8±2 6
Euthyroid	319±4	44 4±1 7
Hyperthyroid	279±3	55 8±1 9

of synthesis of catecholamines in the adrenal medulla have been unable to demonstrate any differences in the hypothyroid (Hutcheon and Parker 1950) or the hyperthyroid animal (Van Arman 1951) Harrison (1961) in a study of thyrotoxic patients found that the release of adrenaline in response to insulin-induced hypoglycemia, as measured by urinary catecholamine excretion, was decreased compared to other published normal values (Euler and Luft 1952) This study was later confirmed by Leak *et al* (1963) In a similar experiment, Leak *et al* (1962) found a decreased response in hypothyroid patients as well From this evidence the authors concluded that a balance in the thyroid status was required for optimal release of catecholamines from the adrenal gland

After the demonstration by Landau (1958) of the hyperglycemia following injection of 2-deoxyglucose (2-DG) in the rat, Hokfelt and Bygdeman (1961) showed that this was accompanied by a marked decrease in the adrenaline content of the adrenal medulla with the corresponding appearance of large quantities of adrenaline in the urine, presumably in response to an intracellular glucopenia of the hypothalamic receptor neurons sensitive to glucose (Dunér 1953) In the present study an attempt has been made to relate the evidence obtained from the above mentioned studies of urinary catecholamine excretion after hypoglycemic stress in hypothyroid and hyperthyroid patients with the earlier studies of the adrenal gland depletion in experimental animals 2-Deoxyglucose has been chosen as the agent because of its ability to release quantities of adrenaline that can be obtained only with convulsive doses of insulin. In addition, the hyperglycemic response evoked by 2 DG provides further simultaneous evidence of the magnitude and pattern of the adrenaline release from the adrenal glands

Methods

Preparations of animals

46 male rats (218—258 g of the Sprague Dawley strain were randomly assigned to 3 groups. 14 animals were made hypothyroid by the daily administration of 0 2 % propylthiouracil in the diet which consisted of a ground well balanced laboratory rat food mixed with an equal volume of tap water 16 animals were made hyperthyroid by daily s c injection of 10 μg 3-tri-iodothyronine (kindly supplied by Smith Kline and French Lab Phil Pa) Including 16 euthyroid control animals all rats received the ground food diet and water *ad lib*, as well as daily injections of either the triiodothyronine 20 μg ml in alkaline saline or alkaline saline alone

Experimental procedure

the rats

1 ml tail

ed s c

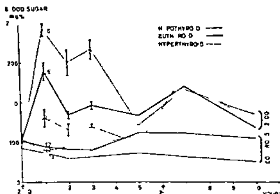


Fig 1 Blood sugar response evoked by s c administration of 2 DG (250 mg/kg b w × 2) in rats Numbers in parentheses represent the number of determinations made in each group for every plotted value Standard errors are plotted for the first 3 hrs

injection of 2 DG 250 mg/kg b w The 2-deoxyglucose (Fluka AG Switzerland) was dissolved

at 2 hr intervals with 1 ml of acetic acid

Analytical methods

Results

appeared to be hyperactive and irritable All of the animals receiving 2 DG displayed symptoms of hypothermia (crouching piloerection, cool and cyanotic extremities etc) most prominently seen in the hypothyroid group

Because of the well known effects of thyroid metabolism on total body weight cardiac size and function and the adrenal cortex the values for catecholamine content or excretion were expressed per animal

The blood sugar curve

The blood sugar response to 2 DG administration seen in all groups is similar to that reported by earlier workers (Landau 1958 Hökfelt and Bydgeman 1961) (Fig 1) However it is apparent that there is a large difference in the response of the 3 groups of animals the hyperthyroid reacting least the euthyroid significantly more and the hypothyroid rats most of all The hyperglycemic response to the second dose of 2 DG is

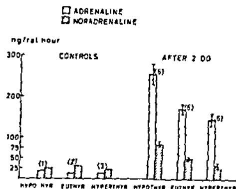


Fig 2

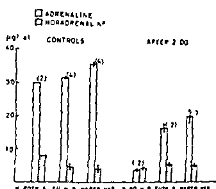


Fig 3

Fig 2 Urinary excretion of catecholamines after s c administration of 2 DG (250 mg kg b w × 2) in rats Numbers in parentheses represent the number of determinations made in each group (The urine of 2 animals was pooled for each determination) Standard errors are plotted for the 2 DG treated groups

Fig 3 Adrenal gland content of catecholamines after s c administration of 2 DG (250 mg kg b w × 2) in rats Numbers in parentheses represent the number of animals in each group Standard errors are plotted for all groups of more than 3 animals

diminished except for the hyperthyroid group and the differences between the 3 groups

groups of control animals are similar and relatively constant

Urinary catecholamine excretion

Examination of the urinary catecholamine excretion after 2 DG reveals a massive increase in the adrenaline excretion of all 3 groups of animals Fig 2 Moreover, the pattern of increased reactivity to 2-DG seen in the blood sugar curve of the hypothyroid animals is reflected in significantly larger amounts of excreted adrenaline compared to either the euthyroid or hyperthyroid animals While the difference in the mean adrenaline excretion of the euthyroid and hyperthyroid rats is not as great as that between them and the hypothyroid rats it seems to agree with the decreased response of the hyperthyroid animals seen in the blood sugar curve The noradrenaline excretion is increased to a lesser extent than adrenaline after 2 DG but reveals the characteristic differences in the 3 groups The urinary catecholamine values from the 3 groups of control animals including the normal euthyroid group appear similar to each other and to normal excretion values obtained with this technique

The adrenal glands

The adrenal glands of the hyperthyroid rats show the characteristic increase in size described by earlier investigators Preston 1928, Feldman 1951 Table I Examination of the catecholamine content of control animals from each group shows little difference in either the amount of adrenaline or noradrenaline Fig 3 The hypothyroid animals

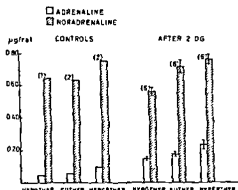


Fig 4 Heart content of catecholamines after s c administration of 2 DG (250 mg/kg b w × 2) in rats Numbers in parentheses represent the number of determinations made in each group (The hearts of 2 animals were pooled for each determination) Standard errors are plotted for the 2 DG treated groups

contain insignificantly larger amounts of noradrenaline, whereas the hyperthyroid animals contain slightly more adrenaline After 2-DG the adrenals of the hypothyroid animals are depleted to less than 13 % of control adrenaline values The euthyroid animals are depleted to 52 %, and the hyperthyroid animals to 56 % of control values No large changes are found in noradrenaline content after 2 DG, but there appears to be a slight decrease in the noradrenaline content of the hypothyroid animals

Heart catecholamines

The heart catecholamine levels of the hypothyroid and euthyroid control rats are similar, both in regard to noradrenaline and adrenaline (Fig 4) The hyperthyroid hearts appear to contain slightly larger amounts of noradrenaline and twice the normal levels of adrenaline After 2 DG the hearts of all 3 groups contain considerably increased absolute and relative amounts of adrenaline, the amount rising from approx 8 to 20 per cent of the total catecholamines The absolute increase in the hyperthyroid animals is greater than in the other 3 groups

Discussion

The above data suggest that there is a quantitative difference in the response of animals to the acute stress of 2-deoxyglucose administration which is dependent on their thyroid status Although the experimental methods employed to produce either hypothyroidism or hyperthyroidism have important limitations the spectrum of the response over the 3 groups seems to indicate that it is most closely associated with the level of thyroid metabolism or the secondary physiological changes which result from different thyroid conditions Since the dose of 2 DG given to each animal was based on its body weight it might be possible to argue that the decreased response of the hyperthyroid animals compared to the heavier euthyroid controls was due to a disproportionately low dose (Table I) However the hypothyroid animals although also smaller in total b w react with an increased response so the method of calculating the dosage seems satisfactory

The results seen in the urinary catecholamines of the hyperthyroid rats stressed with 2 DG are similar to the decreased reactivity obtained after insulin induced hypoglycemia

in thyrotoxic patients (Harrison 1961) However, the increased urinary catecholamine excretion obtained from the hypothyroid rats suggests that there are possibly other mechanisms, such as renal function, which may account for the decreased catecholamine excretion of hypothyroid patients, as studied by Leak et al (1962) Although increased noradrenaline levels are found in rat urine after insulin induced hypoglycemia (Hokfelt and Bygdeman 1961), the increase observed after 2 DG may result from the hypothermia which this agent induces

While no large variations were seen in the catecholamine levels of the adrenal glands from control animals from each group, the changes seen after 2 DG are similar in direction to those obtained by Hökfelt (1951) with insulin With the support of data from the urinary catecholamine excretion and the blood sugar curve, it seems likely that the cause of the different degrees of adrenal gland depletion is based on an increased response in the hypothyroid animals and a decreased response in the hyperthyroid group Even assuming a recovery of adrenaline in urine as high as 8 per cent of that released from the adrenal glands (cf Leduc 1961) and original control levels of approximately 30 μg adrenaline in the adrenals of each rat it is apparent that resynthesis is occuring in all 3 groups of animals at similar and considerable rates (around 1 μg/hr) which agrees with the findings of Hutcheon and Parker (1950) van Arman (1951 and Bygdeman Euler and Hokfelt (1960)

The increased levels of noradrenaline in the hearts of the hyperthyroid control rats is similar to that observed by Goodall (1951) in sheep and Hökfelt (1951) in the rat The increased levels of adrenaline in the hearts of the hyperthyroid control rats agrees with the findings of Leduc Dubreuil and d Iorio (1955) but differs from that of Hokfelt (1951) The markedly elevated adrenaline values in the hearts of the 2 DG treated rats probably result from increased blood levels of adrenaline as indicated by the increased urinary output of adrenaline The higher absolute amounts of adrenaline in the hyperthyroid hearts both in control and 2 DG treated rats as compared to the respective hypothyroid and euthyroid rats could be explained by an increase in the ability of the hyperthyroid heart to take up and retain circulating adrenaline

The findings in the hearts and adrenals of the hyperthyroid rats in this experiment suggest an interesting possible explanation for the well known increase in sensitivity of nearly all kinds of catecholamine receptors in hyperthyroidism It has been shown that procedures such as sympathetic denervation which presumably decrease the amount of noradrenaline reaching receptors result in increased receptor sensitivity The hyper sensitivity of receptors to catecholamines seen in hyperthyroidism could therefore be the result of a similar decrease in the regular release rate or different disposition of catecholamines from both the adrenergic neurons and the adrenomedullary cells

One possible interpretation of the differential response of the adrenal medulla of the 3 groups of animals in this study is that more adrenaline is released from the medulla of the hypothyroid animals and less from the hyperthyroid in response to similar stimuli from the preganglionic secretory nerves relaying impulses from the central nervous system An alternative explanation could be a differential sensitivity of the blood glucose level receptor neurons of the hypothalamus resulting in an increased stimulation in the case of the hypothyroid animals and a corresponding decrease for the hyperthyroid animal This difference in sensitivity may be dependent on the ability of the neurons to maintain normal energy metabolism despite a temporary, acute interference with the first steps of glucose entry and metabolism resulting from the competitive inhibition of the hexokinase reaction see Brown 1962 produced by 2-deoxyglucose

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Influence of Adrenaline on Blood Flow and Metabolism in the Human Forearm

By

LENNART LUNDHOLM and NILS SVEDMYR

Received 31 March 1965

Abstract

Lundholm L and N S [illegible]
forearm Acta ph[illegible]
raised the bloo[illegible]
elimination in t[illegible]
rise in CO_2 production is assumed to have been due to increased oxygen consumption as well as to an increase in the lactic acid content of the tissues The rise in CO_2 tension resulted from an augmented CO_2 production Adrenaline's observed vasodilator effect is attributed at least in part to heighted CO_2 tension of blood and tissues

Experiments on cats have revealed a close association between adrenaline's vasodilator action in skeletal muscle and its stimulation of lactic acid production (Lundholm 1956 The vasodilation was not however directly correlated to that production but rather to an increase in CO_2 production This increase was attributed to neutralization of lactic acid by the tissue bicarbonate, the oxygen consumption of skeletal muscle of the cat did not rise after adrenaline infusion In rabbit experiments a connection was noted between the vasodilator and the lactic acid stimulating effects of isoprenaline Lundholm and Svedmyr 1964)

Corresponding studies in humans have yielded more controversial results Barcroft and Cobbold 1956) found during the later stages of infusion a close association between the blood lactic acid level and the vasodilator effect of adrenaline The latter substance they reported had a biphasic effect on the blood flow of the forearm — an initial rise and a sustained rise While the sustained rise in their view, was due to increased lactic acid production the initial rise had a different mechanism De la Lande *et al* (1961) found that adrenaline raised the lactic acid content of venous blood from the muscle and demonstrated a stimulatory effect on the carbohydrate metabolism of human muscle Such an effect had previously been doubted since

Hildes, Purser and Sherlock (1949) had failed to detect any glycogenolytic action of adrenaline in human skeletal muscle on i a infusion Allwood and Cobbold (1962) observed selective inhibition by phenoxybezamine of adrenaline's vasoconstrictor effect in the human forearm the vasodilator action was intensified and the stimulation of carbohydrate metabolism was not inhibited De la Lande and Whelan (1962) studied the influence of sodium lactate and lactic acid solution at pH 3, administered by i a infusion upon the forearm blood flow, but were unable to demonstrate any vasodilator action

In the abovementioned investigations on human subjects the effect of adrenaline was studied only upon the lactic acid content in mixed venous blood or in venous blood from the relevant muscle In experiments on cats (Lundholm 1956) it was found, however, that i a infusion of lactic acid so raised the muscle blood flow that the lactic acid content of venous blood from the muscle sometimes fell below the basal level Only when lactic acid elimination with the blood was calculated from the product of the venous arterial lactic acid difference and the blood flow was it possible to demonstrate an increase The elimination of lactic acid with the blood, however, did not accurately reflect the rate of lactic acid formation in the muscle Lactic acid diffuses rather slowly in muscle tissue (Eggleton, Eggleton and Hill 1928, Sacks and Sacks 1937), hence, the elimination did not increase immediately and the content in the muscle rose Lactic acid elimination was preceded by an increase of CO_2 elimination commensurate with that of the lactic acid content The increased blood flow after adrenaline was not considered a direct effect of lactic acid, it was related to an elevated CO_2 production and CO_2 tension in the tissues

In these circumstances we deemed it worth while to investigate if there was a relationship between adrenaline s metabolic effects and its vasodilator action in the human forearm To this end we studied the effects of intravenously infused adrenaline upon the forearm blood flow, lactic acid elimination, oxygen consumption and CO_2 production, as well as the CO_2 tension, pH and lactic acid content of arterial blood and of venous blood from the forearm muscles Since adrenaline may raise the ATP content of skeletal muscle (Mohme Lundholm and Svedmyr 1965), and ATP itself has a powerful vasodilator action the influence of adrenaline upon the plasma ATP and ADP levels was also investigated

Methods

The experiments were performed on 6 healthy male volunteers 20—25 years of age who had fasted since the previous evening With the subject reclining in a room having a controlled temperature of 25°C a fine teflon catheter was introduced under local anesthesia into the left brachial artery Via the catheter arterial blood samples were taken for determination of O_2 and [illegible]

Grass volume transducer (PT 5 V) on a Grass polygraph Each subject received before the experiment 1 ml 5 per cent Heparin® intravenously

The blood flow of the forearm was first recorded at 1 min intervals for a basal 30-min period during which either one or two basal samples of arterial and venous blood were taken Adrenaline was then infused i v in a dose of 0 1 μg kg/min for 30 min The solution was diluted

TABLE I Effect of adrenaline infusion on blood flow, O_2 consumption, CO_2 production lactic acid elimination and CO_2 tension of human forearm Mean of 6–8 tests ± S E M

		Increase over basal values		
		Duration of infusion min		
	Basal values	3	10	30
Blood flow *ml/100 ml tissue/min*	2 50 ± 0 35	2 33 ± 0 39 p<0 001	3 16 ± 0 72 p<0 01	4 01 ± 0 77 p<0 01
O_2 consumption ml/100 ml tissue/min	0 144 ± 0 025	0 040 ± 0 01 p<0 01	0 070 ± 0 022 p<0 02	0 059 ± 0 013 p 0 01
CO_2 production ml/100 ml tissue/min	0 093 ± 0 021	0 105 ± 0 024 p<0 01	0 023 + 0 015	0 056 + 0 026
Lactic acid elimination mg/100 ml tissue/min	0 014 ± 0 011	0 040 ± 0 037	0 081 + 0 030 p 0 05	0 229 ± 0 077 p<0 05
pCO_2 arterial blood mm Hg	34 4 ± 2 1	1 5 ± 0 7	2 4 + 0 8 p 0 05	1 6 ± 0 13 p<0 05
pCO_2 venous blood mm Hg	36 5 ± 1 9	3 7 ± 1 0 p<0 02	2 4 ± 1 0 p 0 05	2 7 ± 1 0 p 0 05

with 0 9 *per cent* NaCl *to which 0 1* per cent ascorbic acid had been added *to stabilize the* adrenaline Both arterial and venous blood samples were taken 3 10 30 and 90 *min after the* start of adrenaline *infusion* in addition arterial samples were taken at 20 40 50 60 70 and 80 min

[illegible]

arterial *differences and the means of the blood flow values obtained before and after the withdrawal of blood samples*

Results

Blood Flow The effect of i v infused adrenaline (0 1 µg kg min on the blood flow of the forearm is evident from Table I and Fig 1 During infusion the blood flow gradually rose and reached approximately double the basal level Following infusion the flow slowly decreased and reached the preinfusion level within some 30 min In some experiments a biphasic effect was noted the blood flow *first rising substantially during the first few minutes then falling towards the basal level then gradually rising once* more A trace of this biphasic effect can be seen in the mean curve (Fig 1)

Lactic Acid Elimination Under basal conditions venous blood from the forearm generally had a somewhat higher lactic acid content than did arterial blood (Fig 2), hence there was some degree of lactic acid elimination *In some experiments* however

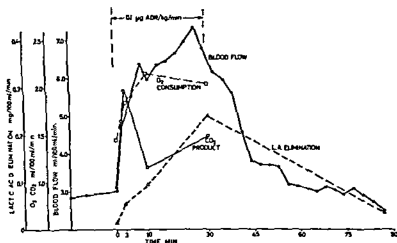


Fig 1 Effect of intravenously infused adrenaline on blood flow, oxygen consumption, CO_2 production and lactic acid elimination in human forearm Each point is the mean of 6—8 tests

when the basal lactic acid content of arterial blood was elevated it exceeded that of the venous blood, hence the forearm muscles were extracting acid from the blood The maximum extraction amounted to 0 06 mg/100 ml muscle/min

Three min after the start of adreanline infusion the lactic acid content of both venous and arterial blood was unchanged (Fig 2) This notwithstanding, lactic acid elimination had risen since the blood flow was increased After adrenaline infusion for 10 and for 30 min both the venous and arterial blood lactic acid levels and the venous arterial lactic acid difference were increased as was lactic acid elimination The increase was significant both after 10 and after 30 min (Table I) 60 min after adrenaline infusion had ceased lactic acid elimination still was higher than the pre-infusion level

Oxygen Consumption After only 3 min adrenaline infusion the oxygen consumption had risen in the forearm (Fig 1 Table I) The elevation persisted, with little change, throughout the infusion and amounted to about 40 per cent of the basal value

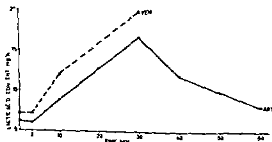


Fig 2 Effect of adrenaline on lactic acid content of venous blood from the forearm muscles (ven) and of arterial blood (art Adrenaline was infused in a dose of 0 1 μg kg min during the period from 0 to 30 min Each point is the mean of 6—8 tests

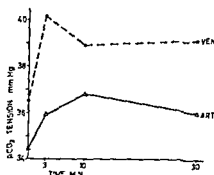


Fig 3 Effect of adrenaline on plasma CO_2 tension for arterial blood (art) and for venous blood from the forearm muscles (ven) Each point is the mean of 6 tests

Carbon Dioxide Production At the end of 3 min adrenaline infusion the CO_2 production was more than doubled, and the increase was statistically significant (Fig 1, Table I This initial rise was considerably greater than that of oxygen consumption The respiratory quotient rose from the basal value of approximately 0 70 to 1 1 at the 3 min mark When determined 10 and 30 min respectively after the start of adrenaline infusion the increase in CO_2 production was more moderate, being commensurate with an elevated oxygen consumption at an unchanged respiratory quotient If the mean effects after 10 and 30 min was calculated together the increase over the basal value was 0 037 ± 0 010 ml/tissue/min The difference being statistically significant ($P < 0.01$)

Carbon Dioxide Tension During infusion of adrenaline the CO_2 tension rose both in arterial and in venous blood (Table I Fig 3) For venous blood the rise was most pronounced at the end of 3 min infusion when the CO_2 production too was maximum

Plasma pH Neither arterial nor venous plasma pH changed appreciably during infusion of adrenaline For arterial blood the basal pH averaged 7 438, the corresponding value after 30 min infusion was 7 443 For venous blood the plasma pH was 7 433 *initially and 7 430 on completion of the infusion*

Adenylic Acid Compounds In 3 expts the plasma ATP ADP and AMP levels were determined before and during the infusion of adrenaline No changes were noted

Discussion

Adrenaline stimulated the blood flow oxygen consumption CO_2 production and lactic acid elimination in the human forearm and raised the lactic acid content and CO_2 tension of the blood Some of these effects were probably correlated

Blood Flow In a few experiments the blood flow showed a typical biphasic effect i e first a substantial rise then a decline towards the initial level then once more a gradual increase The increase persisted long after completion of the infusion The mean blood pressure was not changed during the infusion of adrenaline (Lundholm and Svedmyr 1966 a The increase in the blood flow was therefore attributed to vasodilation and not to heightened perfusion pressure

The vasodilating effect of adrenaline in skeletal muscle is thought to depend on stimulation of adrenergic β receptors As pointed out by Lundholm (1956 and recently confirmed by Grant 1964 isolated vessels from skeletal muscle are only contracted

by local application of adrenaline in all concentrations I v injection of adrenaline however, dilate the same vessels

These facts indicate that the β receptor mediating dilatation in skeletal muscle are probably situated in the striated muscle cells The vasodilating effect of adrenaline is thereby thought to be caused by a metabolic action Both the increased oxygen consumption and lactic acid production of the forearm were probably of importance for the vasodilating effect of adrenaline

Oxygen Consumption Adrenaline increased the oxygen consumption in the forearm by approximately 40 per cent This particular calorigenic action of adrenaline has not previously been recorded in humans In experiments on cats (Lundholm 1956) adrenaline tended to depress oxygen consumption in the muscles Euler (1931), however, reported that adrenaline increased the oxygen consumption of skeletal muscle in dogs Bearn Billing and Sherlock (1951) found that adrenaline raised the oxygen consumption of human liver, coincident with an increase in lactic acid extraction and blood flow Adrenaline's calorigenic action was thought to be attributed to increased oxidation of lactic acid Our results indicate that in man a calorigenic response to adrenaline also occurs in skeletal muscle Its mechanism is not known One possibility is that the lactic acid increment produced by adrenaline was accompanied by increased oxidation of lactic acid in the muscle The extraction of lactic acid noted in some experiments on the forearm might favour this view On the other hand the calorigenic action of *noradrenaline* did not depend on its ability to raise the lactic acid content of the blood (Lundholm and Svedmyr 1965 b) There is reason therefore, to assume that other mechanisms besides increased oxidation of lactic acid may be involved in the calorigenic action of catechol amines in man The subject is discussed by Lundholm, Mohme—Lundholm and Svedmyr (1966)

[illegible]

peripheral resistance In cold adapted rats noradrenaline increased the oxygen consumption up to 100 per cent and decreased the systemic resistance

Carbon Dioxide Production and Lactic Acid Elimination Carbon dioxide and lactic acid are two of the acid metabolites which are thought to be implicated in metabolic vasodilatation In the present experiments the CO_2 production rose during adrenaline infusion, but the rise was maximal three min from the start, when lactic acid elimination had only risen slightly This peak in CO_2 production is not likely to have been due solely to an increase of oxidative metabolism Nor is it very probable that adrenaline would have a relatively long latent period in stimulating the lactic acid production of muscle A more plausible hypothesis is that the stimulation was maximal or almost maximal from the outset of infusion but since lactic acid diffuses slowly in muscle tissue (Eggleton Eggleton and Hill 1928) an accumulation occurred in the muscles, and the acid was neutralized by the tissue bicarbonate The estimated magnitude of this extra peak in CO_2 production is consonant with such a view

The increment of CO_2 production over and above that which could reasonably be ascribed to increased oxygen consumption was naturally dependent on the respiratory quotient Assuming that the latter did remain unchanged during the infusion — it averaged 0 70 prior to infusion and 0 73 after 30 min — the extra CO_2 production

would have amounted to 0 105—0 7 0 039 or 0 077 ml/100 ml *tissue/min or 3 4 µmoles per min* This amount of CO_2 is equivalent to 0 31 mg lactic acid/100 ml tissue/min — a value only slightly exceeding the maximum *increase of lactic acid* elimination (0 23 mg/100 ml tissue/min) recorded after 30 min infusion Since adrenaline also raised the contents of other organic acids in the muscle — *pyruvic acid, hexophosphoric acids — a somewhat higher value might have been expected*

If adrenaline did increase the muscle lactic acid production by about 0 23 mg/100 ml tissue/min, the increment was less than that noted in cats which amounted to approximately 1 mg/100 g muscle/min (Lundholm 1956) This value, however, was referable purely to muscle, whereas in the experiments on human forearm the tissues included skin and bone If these together constituted 50 per cent of the total tissue, and were not involved in the formation of lactic acid, the lactic acid production in man would have amounted to same 0 5 mg/100 g muscle/min — i e, one half of that in the cat On intra-arterial infusion of lactic acid in cats the threshold dose for a vasodilator action was in the region of 0 3 mg/100 g muscle/min It seems reasonable therefore, to assume that if human and feline muscle blood vessels were equally responsive to lactic acid, the amount of lactic acid released via adrenaline in man would have had a vasodilator effect However, to duplicate the effect of adrenaline on lactic acid *production by infusion of lactic acid in humans would require infusion of approximately* 10 mg of lactic acid (1 2 ml 0 1 N) per min into the brachial artery, which could probably lead to complications De la Lande and Whelan (1962) infused lactic acid solutions of pH 3 3 (about 0 0003 N) in the brachial artery The lack of vasodilator action in these experiments is probable attributed to the fact that the amount of lactic acid infused was too small

The CO_2 tension rose, both in arterial and in venous blood, after adrenaline The rise was probably attributable both to increased oxygen consumption and to stimulation of lactic acid production in the muscle The elevated CO_2 tension may account in part for adrenaline's vasodilator action The maximum elevation at the end of 3 min coincided with the initially pronounced increase of blood flow noted in some experiments During the later stages of infusion however, the blood flow rose while the CO_2 tension remained fairly stable It appears likely that an additional vasodilator factor came into play during these stages when the lactate of the blood was conspicuously to the blood flow Under certain conditions sodium lactate may have vasodilator properties (Lundholm 1956)

The relative significance of increased CO_2 tension and of the lactate concentration with respect to adrenaline's vasodilator action in the human forearm is a moot question In cat the vasodilating effect of adrenaline in skeletal muscle seems according to Gosselin 1962 to be connected with an increased CO_2 tension The experiments here reported suggest that adrenaline's metabolic effect is a factor in its vasodilator in man This view is supported moreover by the observation that nethalide, which blocks adrenergic β receptors, simultaneously inhibited the metabolic and vasodilator effects of adrenaline in the human forearm Lundholm and Svedmyr 1965

The authors are grateful for the technical assistance of Mr Andras Besz Miss Inga Johansson and Mr Nandor Vamos Financial support has been given by the Swedish State Medical Research Council and the Swedish National Association against Heart and Chest Diseases

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Acta physiol. scand. 1965. 65. 352—356

From the Department of Pathology, Section II, University of Helsinki, Finland

Tissue Eosinopenia in the jejunal Villi of the Isolated Loop during Resorption of Histamine, Acetylcholine and Adrenaline

By

TOIVO RÄSÄNEN

Received 6 April 1965

Abstract

Räsänen, T. *Tissue eosinopenia in the jejunal villi of the isolated loop during resorption of histamine, acetylcholine and adrenaline.* Acta physiol. scand. 1965. 65. 352—356. — Isotonic sodium chloride, isotonic potassium chloride, acetylcholine, histamine, adrenaline and dexamethasone were injected into the loop of the rat jejunum isolated with 2 ligatures. The circulation of the loop was intact. After an hour specimens were taken from the isolated and intact jejunum for the count of tissue eosinophilic leucocytes and of the mucosal mast cells. Sodium and potassium chloride and dexamethasone did not cause any changes in the superficial tissue eosinophils of the lamina propria at the tip of the villi or in the basal tissue eosinophils of the lamina propria. Superficial tissue eosinophilia decreased during the action of acetylcholine (P 0.01) and histamine (P 0.02), less markedly during the adrenaline effect. Basal tissue eosinophils increased in the free intestinal loop during the influence of the last mentioned amine (P 0.01). No changes occurred in the mucosal mast cell counts. It is suggested that eosinophilic leucocytes participate in the detoxication process with their peroxidase enzyme stores and are destroyed.

Metabolites with a direct eosinolytic effect appear in the plasma during stress (Hanes *et al.* 1953). Emotional stress causes eosinopenia in man (Dreyfuss and Feldman 1952, Kerr 1956). The eosinopenia of neurolabile dogs is greater than that of neurostable on account of the effect of infection and ACTH (Burdina and Melikhova 1961). Glucose and protein metabolites seem to have an influence which leads immediately to blood eosinopenia (Aschkenasy 1957), differing thus from the eosinopenic effect of corticoids which follows after the initial rise. Eosinopenia in the blood caused by muscle work is more pronounced than metabolism corresponding to the product of energy would suggest, possibly because of neural factors (Yanagihara 1958).

Acetylcholine inhibition with atropine causes an increase in the tissue eosinophilia of rat gastric mucosa (Teir *et al.* 1955). When acetylcholine, histamine and adrenaline are metabolised and rapidly inactivated they possibly consume the peroxidase of eosinophilic

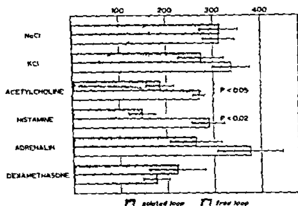


Fig 1 The columns indicate means ± S E of eosinophils counted from 8 test animals in each group

leucocytes In order to study this question, tissue eosinophilia was determined in lamina propria limited to a loop of the small intestine after the short term effect of an injection of the above agents For the sake of comparison a glucocorticoid dexamethasone, with a very marked tissue eosinopenic effect (Rasanen 1962) was included in the study

Method

About 15– 18 cm of the jejunum was isolated with 2 ligatures under a light nembutal-ether anesthesia in 4 month-old male rats of Dawley-Sprague strain from which both food and drink had been withheld for 12 hrs before the experiment The ligatures were placed so that they did not interfere with the circulation in the loop of the intestine and 2 ml of the solution under examination was injected into the isolated loop The small laparotomy wound was close After 1 hr the abdomen was opened again under ether anesthesia and specimens were taken from the isolated intestinal loop and from the free loop about 3 cm aborally from the distal ligature The specimens were fixed in Bouin s solution and in 4 % fresh basic lead acetate After paraffin treatment sections 4 μ in thickness were cut from the specimens and stained with hemalaun eosin for the determination of eosinophils The samples fixed with lead acetate were cut into thicknesses of 10 μ and stained with toluidine blue for the mast cell count

Eosinophilic leucocytes were calculated separately from the tip of the villi per 20 visual fields and from the basal part of the mucosa also per 20 visual fields. At the tip of the villi the superficial eosinophils were counted if the lamina propria of the villus filled the entire visual field or half of it (oil immersion magnification × 1000 and Leitz plane system) In the basal [illegible]

2 Isotonic KCl aqueous solution
3 Histamine diphosphate 1 mg 2 ml (3 26 × 10 ³ meq of histamine
4 Acetylcholine chloride solution 1 mg 2 ml (5 50 × 10 ³ meq of acetylcholine
5 Adrenaline chloride 1 mg 2 ml (5 30 × 10 ³ meq of adrenaline
6 Dexamethasone-Na phosphate 10 mg/2 ml (20 25 meq of dexamethasone

The solvent in groups 3—6 was isotonic saline Fisher s Student t test was used in the statistical treatment

Acta physiol. scand. 1965. 65. 352—356

From the Department of Pathology, Section II, University of Helsinki Finland

Tissue Eosinopenia in the jejunal Villi of the Isolated Loop during Resorption of Histamine, Acetylcholine and Adrenaline

By

TOIMI RÄSÄNEN

Received 6 April 1965

Abstract

Rasanen T *Tissue eosinopenia in the jejunal villi of the isolated loop during resorption of histamine acetylcholine and adrenaline* Acta physiol scand 1965 65 352—356 — Isotonic sodium chloride isotonic potassium chloride acetylcholine histamine adrenaline and dexamethasone were injected into the loop of the rat jejunum isolated with 2 ligatures The circulation of the loop was intact After an hour specimens were taken from the isolated and intact jejunum for the count of tissue eosinophilic leucocytes and of the mucosal mast cells Sodium and potassium chloride and dexamethasone did not cause any changes in the superficial tissue eosinophils of the lamina propria at the tip of the villi or in the basal tissue eosinophils of the lamina propria Superficial tissue eosinophilia decreased during the action of acetylcholine (P 0 05) and histamine (P 0 02) less markedly during the adrenaline effect Basal tissue eosinophils increased in the free intestinal loop during the influence of the last mentioned amine (P 0 01) No changes occurred in the mucosal mast cell counts It is suggested that eosinophilic leucocytes participate in the detoxication process with their peroxidase enzyme stores and are destroyed

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Metabolites with a direct eosinolytic effect appear in the plasma during stress (Taylor *et al* 1953) Emotional stress causes eosinopenia in man (Dreyfuss and Feldman 1952 Kerr 1956) The eosinopenia of neurolabile dogs is greater than that of neurostable on account of the effect of infection and ACTH (Burdina and Melikhova 1961) Glucose and protein metabolites seem to have an influence which leads immediately to blood eosinopenia (Aschkenasy 1957) differing thus from the eosinopenic effect of corticoids which follows after the initial rise Eosinopenia in the blood caused by muscle work is more pronounced than metabolism corresponding to the product of energy would suggest, possibly because of neural factors (Yanagihara 1958)

Acetylcholine inhibition with atropine causes an increase in the tissue eosinophils and rat gastric mucosa (Teir *et al* 1955) When acetylcholine histamine and adrenaline are metabolised and rapidly inactivated they possibly consume the peroxidase of eosinophilic

Acetylcholine disintegrates very rapidly in the tissues under the influence of esterase and the metabolisation of histamine in the tissues also results very rapidly in its inactivation (Halpern, Neveu and Wilson 1959) It is possible that the rapid destruction of acetylcholine and histamine takes place in the villi as adrenaline may be resorbed into the organism and cause a persisting decrease in the tissue eosinophilia of the isolated intestinal loop in contrast to increased basal eosinophilia in the intact intestinal loop In the gastric mucosa atropinisation leads to an increase in tissue eosinophilia (Teir *et al* 1955) and inhibits the disintegration of acetylcholine in the cerebral cortex (MacIntosh and Oborin 1953)

Histamine produces an immediate drop in blood eosinophilia (Rose and Brown 1938, Panzenhagen and Speirs 1953) and eosinophilic granules inhibit the effect of histamine on smooth muscle (Vercauteren 1955) Histamine is destroyed in the mucosa of the rat intestine, this effect is inhibited after enterectomy (Millican 1953) The peroxide that forms in the amine acetylisation process splits peroxidatically in the organism Peroxide forms stoichiometrically when adrenaline disintegrates under the influence of monoamino-oxidase which is profuse in the intestinal mucosa (Aebi 1961)

Intestinal mucosa contains numerous eosinophilic leucocytes and hence a considerable peroxidase activity (Teir and Rytomaa 1961, Undritz 1952, Vercauteren 1955) The eosinophilic reaction established in the villi is probably caused by consumtion of the peroxidase needed for the detoxication reactions and by the concurrent destruction of eosinophilic leucocytes

It has been observed that normally eosinophil leucocytes in rat after partial loss of the myeloperoxidase, migrate through the intestinal epithelium in the lumen (Teir *et al* 1963 Teir and Rytomaa 1965) In the lamina propria of the isolated jejunal loop the loss of the eosinophilic peroxidase may occur violent during the destruction of additional acetylcholine and amines

Dexamethasone causes eosinopenia in the gastric mucosa after repeated injections (Rasanen 1962) as cortisone does in the intestinal mucosa (Sundell 1958) No changes seem to occur in the eosinophilia of the intestinal mucosa in an hour A single cortisone injection causes an increase in eosinophilia in the intestinal mucosa of the rat, especially in atropinised rats (Sundell 1958)

The autonomous nervous system may contains much histamine compared with the spinal nerves (Euler 1956) possibly bound to mast cells (Torp 1961) Adrenaline and acetylcholine are released from the nerves during stimulation The reactions that follow for their inactivation may be the cause of eosinopenia in stressed test animals (Tanos *et al* 1953) or when neural tension is emphasized (Dreyfuss and Feldman 1952, Kerr 1956 Yanagihara 1958)

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From the Histological Departments University of Uppsala and Karolinska Institutet Stockholm, Sweden

Fluorometric Assays of Glutamic-Pyruvic Transaminase Activity in Microdissected Pancreatic Islets from Obese-Hyperglycemic Mice

By

Bo Hellman

Recieved 8 April 1965

Abstract

... insulin secretion supports the view that transamination processes represent an important metabolic feature in the maintenance of the specific endocrine function of the β cells

... amino acids may be synthesized from this stage by *transamination and secondary transformations* of the primary amino acids

With the introduction of a procedure for large scale isolation of mammalian islets of Langerhans it became possible to extend our analyses of the transamination mechanisms

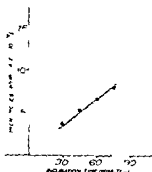


Fig 3

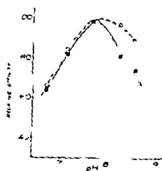


Fig 4

Fig 3 Relation between the incubation time (minutes) and the measured GPT activities in an islet homogenate

Fig 4 Relative GPT-activities at different pH values The enzyme activities were expressed in per cent of the maximum activities measured in the islet (——■——) and exocrine pancreatic tissue (— — — —)

micromoles of DPN in the sample solution Serial dilutions of the different tissue homogenates were used for studying the relation between the enzyme concentration and the amount of pyruvate formed during the present assay conditions In the latter case a linear relationship was obtained up to about 20 10 ³ μmoles pyruvate (Fig 1), which corresponds to a 16 % consumption of the DPNH in the incubation medium This means that a dry weight of about 25 μg islets and 4 μg exocrine parenchyma or liver tissue represents the maximal amounts, which could be assayed for GPT in the obese-hyperglycemic mice

2 *Substrate concentrations* The influence of the concentration of each of the substrates L-alanine and α ketoglutarate was tested separately The relation between the islet enzyme activity and the concentration of the substrates can be seen in Fig 2 It is evident that both the concentrations of L-alanine (41 mM and α ketoglutarate (7 mM) used in the standard procedure fell on the horisontal part of the curves

3 *Dissection and incubation time* The possibility that enzyme leakage or inhibition during the dissection procedure would have influenced the results was tested by recording the GPT-activity in specimens of endocrine and exocrine pancreas kept for different periods of time in the dissection bath For a dissection time up to 30 min no significant effects were noted The influence of the incubation time on the enzyme activity is shown in Fig 3 The 45 min incubation at 37 C used in the standard procedure could be considerably extended without deviation from a linear relationship

4 *pH optimum* The transaminase activity was determined at different pH values of the Tris buffer Both the endocrine and exocrine pancreatic homogenates displayed a maximum within the pH range of 7 5 8 0 Fig 4 The decrease of the transaminase activity with higher pH values tended to be more pronounced in the islet tissue than in the exocrine parenchyma

5 *Effect of pyridoxal phosphate* Omission of pyridoxal phosphate (4 mg %) from the incubation medium did not decrease the GPT-activity measured in the islets or in the other tissues either for the NO or NOS-mice

TABLE III The relative GPT-activities recorded for various concentrations of carbutamide in the incubation medium (the values obtained in the absence of sulfonylurea were denoted as 100) The figures represent the mean values of 4 separate experiments based on material from 2 AO and 2 AOS mice

	Relative GPT activity		
Carbutamide mg %	Islets	Acinar tissue	Liver
2 5	111	102	99
25 0	95	97	93
250 0	77	69	55

B Enzyme activities

0 07 MKH units was noted as compared with 3 42 ± 0 36 for the exocrine parenchyma ($t = 8\ 74$, $P < 0\ 001$) and 5 15 ± 0 73 for the liver ($t = 7\ 37$ $P < 0\ 001$) While the starvation period induced a 40 % reduction of the islet GPT activity ($t = 3\ 30$ P <

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homogenates particularly ... free access to food In the presence of 25 or 250 mg % of this compound there was on the other hand an enzyme inhibition also in the pancreatic islets

Discussion

The GPT activity was measured with a fluorometric procedure which is similar in principle to that described by Wroblewski and LaDue (1956) except that instead of measuring spectrophotometrically the disappearance of DPNH the DPN formed is measured fluorometrically After a systematic study of the possibilities of using the latter alternative for measuring GPT in serum Laursen and Hansen (1958) reported the fluorometric procedure to give very reproducible results The small amounts of tissue represented by the mammalian islets of Langerhans made it necessary to make use of the high sensitivity offered by fluorometric assays in a DPN DPNH system In our analyses of the optimal assay conditions the usefulness of the fluorometric approach has been fully confirmed also for tissue homogenates In the obese hyperglycemic mice a dry weight of up to 25 μg islets and 4 μg exocrine pancreas or ... alent to ... fluores ... s on the ... er after ... ings do

... tion The latter

Acta physiol. scand. 1965. 65. 364—369

From the Department of Physiology, Karolinska institutet, Stockholm, Sweden

The Tactile Hairs on the Cat's Foreleg

By

Bengt Y. Nilsson and C. R. Skoglund

Received 13 April 1965

Abstract

Nilsson, B. Y. and C. R. Skoglund. *The tactile hairs on the cat's foreleg.* Acta physiol. scand. 1965. 65. 364—369. — The vibrissae-like hairs at the wrist of the cat's foreleg have been studied morphologically and physiologically. Each hair follicle contains a large blood sinus close to which a number of Pacinian corpuscles are situated. Recording from nerve filaments allowed most nerve endings to be spontaneously active. Bending of a hair resulted in an increased discharge frequency, up to [illegible]00 per sec, rapidly declining to a steady frequency level of 20—40 per sec which was maintained without appreciable further decline during several minutes. Bending in the opposite direction might cause cessation of the spontaneous discharge but otherwise no pronounced directional sensitivity was observed. Releasing the hair was usually followed by a silent period in the spontaneous discharge. Contrasting with the slowly adapting discharges of the hair endings are the transient responses of the Pacinian corpuscles with characteristically low threshold to vibratory stimulation. The significance of the blood sinus and of the closely related Pacinian bodies is briefly discussed.

Hairs with a specific tactile function are found in many mammalian species. They are most highly developed in carnivora and rodents, i.e. animals which for their nocturnal foraging expeditions are dependent on a keen perception of touch. These tactile hairs — which differ from the ordinary covering hairs of the body in that the capillary network of the follicle has developed into a blood sinus — are as a rule located around the mouth, the so-called vibrissae.

Bland Sutton (1887) observed a tuft of long and stiff hairs at the wrist of certain Lemurs and later Beddard ([illegible]) described a tuft of vibrissae-like hairs distally on the forelimb in many other mammalian species. The occurrence of these hairs seems to vary in different species but they are above all to be found in animals using the forelimbs to grasp or feel.

The microscopical anatomy of the vibrissae has been the subject of a great number of investigations ever since the 19th century (see e.g. Vincent 1913, Melaragno and Montagna 1953), but no detailed microscopical description of the carpal tactile hairs seems to have been published so far. Frédéric (1905) mentions, however, that the follicles of carpal hairs from the species *Hapale* [illegible]

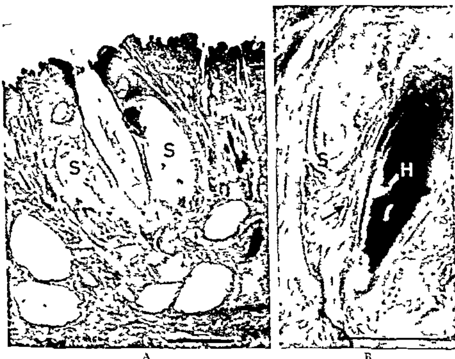


Fig 1 A Longitudinal section of hair follicle with blood sinus (S) and five Pacinian corpuscles Black line 0.5 mm

Fig 1 B Longitudinal section of hair (H) and blood filled sinus (S At arrow nerve fibers passing through cavernous part of sinus Black line 0.5 mm

In connection with other investigations on the cat s forelimb (Skoglund 1960 a great number of Pacinian corpuscles were observed round the blood sinuses of the carpal hairs This finding prompted a histological study of these hair structures, the results of which are reported in the first part of the present paper

The second part of the paper gives an account of recordings made from nerve filaments from this structure showing that the physiological properties of the carpal hairs closely resemble those demonstrated for vibrissae (Fitzgerald 1940), their chief characteristics being slowly adapting end organs while other rapidly adapting impulse responses can be shown to derive from Pacinian corpuscles The results were briefly reported at the XI Scandinavian Physiological Congress (Nilsson and Skoglund 1963)

Morphology

On the cat s forelimb just above the wrist on the volar side there is a small tuft of 3—6 rigid hairs of a length of 1—2 cm which protrude from an easily palpable elevation in the skin This elevation which is of a pale bluish lustre is also covered by ordinary hairs (the tactile hairs often differ in colour from the surrounding hairs) It is connected to the underlying fascia by a strong connective tissue band and supplied by a relatively large nerve trunk running together with the blood vessels

In some animals the elevation was dissected free and after fixation in formalin sectioned and stained according to the method of van Gieson or Palmgren.

of a length of 0 7 mm enclosed by a small arteriolar loop could be dissected free and it was found that the pulsations of the loop caused a dislocation of the corpuscle of 15 μ Pulse synchronous impulses could also be recorded from a filament containing the fiber from this corpuscle, and the same unit could also be activated by manual dislocation of the corpuscle by a probe

Discussion

The cat's carpal tactile hairs exhibit properties which closely resemble those of the vibrissae (Fitzgerald 1940), slowly adapting receptors being involved in both cases but there seem to be certain functional differences Thus Fitzgerald estimated the number of spontaneously active endings to be less than 5 % of the total number studied whereas in the present investigation most of the units tested were spontaneously active Fitzgerald observed a marked difference in discharge frequency when the hair was bent in different directions and proposed that this directional sensitivity might be of use in postural adjustments of the head in relation to solid objects In the carpal hairs no pronounced directional sensitivity of this type has been observed but then this may also be of less importance in the paw than in the head

The silent period in the spontaneous discharge on release of a hair may of course be due to mechanical rebound phenomena causing a transient unloading of the receptors However, it may also be a sign of a reduced excitability analogous to that observed in other sensory endings and this concept gains support from the facts that the duration of the silent period is dependent on the duration of the stimulation, and that after a silent period of some length the original frequency is only slowly resumed

Slowly adapting mechanoreceptors in so-called "touch spots" in the cat have been studied by Iggo (1962) Pressure applied at such a spot resulted in an impulse discharge of slowly diminishing frequency lasting more than 5 minutes There is a striking similarity between the adaptation patterns of these "touch spots" and of the carpal tactile hairs In view of this similarity it is of special interest that according to Iggo the "touch spots" are very richly vascularized with "a whorl or glomus of small blood vessels probably capillaries which were always filled with a rich flow of blood"

The presence of a large blood sinus in the root of the hair has been the object of a great deal of speculation in the earlier anatomical literature Messenger (1900) was of the opinion that the sinus may act as a hemostatic apparatus by which the sensitivity of the hair can be graded Vincent (1913) pointed out that the free suspension of the hair in the sinus makes it possible for vibrations of a near by object to be transmitted to the vibrissae, which would not be possible if the hair was fixed in an unyielding tissue She also mentioned that the stimulus threshold of the receptors might perhaps be raised or lowered by pressure changes in the sinus Vincent as well as also Potter and Charpy (1912) discussed a possible reflex mechanism which might influence the sensitivity of the hair via contraction of the musculature compressing the blood sinus and dampening the vibrations of the hair

No experimental study of the significance of the blood sinus seems to have been made However, Fitzgerald (1940) investigated the effect of different ions injected intra arterially into the cat's vibrissae bearing area and found that the spontaneous discharges as well as the rate of adaptation of the evoked discharges were dependent on the ion concentration It is possible that the large amount of blood continually flowing around

the nerve endings in the tactile hair contributes to the maintenance of a constant chemical milieu which may be a prerequisite for the slow adaptation of these receptors

Gammon and Bronk (1935) showed that the Pacinian corpuscles in the mesentery are sensitive enough to respond to pulsations in the mesenteric vessels, and pulse synchronous discharges were observed also in the present investigation The close connection of Pacinian corpuscles to the blood sinuses of the carpal tactile hairs raises the question of their functional relation to this circulatory system and this comparatively easily accessible preparation may open up a possibility to study this question

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Acta physiol. scand. 1965. 65. 370—377

From the Neurophysiological Laboratory at the Anatomical Institute, University of Oslo, Norway

Peripheral Autonomic Influence on the Motility of the Urinary Bladder in the Cat

I. Rhythmic Contractions

By

Ragnar Gjone

Received 28 April 1965

Abstract

Gjone, R. *Peripheral autonomic influence on the motility of the urinary bladder in the cat. I. Rhythmic contractions.* Acta physiol. scand. 1965. *65.* 370—377. — In the present work the influence of each peripheral autonomic component was studied by comparing the intravesical pressure curves obtained under progressive bladder denervation in cats lightly anesthetized with nembutal. Intravesical pressure recording was established by the direct approach described in a preced [illegible]

In other experiments the sympathetic nerves were first sectioned bilaterally with the parasympathetic fibres intact. This was followed by a marked enhancement of the frequency and amplitude of the rhythmic bladder contractions. After additional section of the parasympathetic bladder nerves the vesical hyperactivity was abolished. Fairly normal pressure fluctuations were again recorded, similar to those described above from the fully intact bladder. Thus definite evidence of a dual autonomic influence on the rhythmic vesical activity was established by the demonstration of excitatory parasympathetic and inhibitory sympathetic effects.

When fluid is introduced into the bladder cavity, the vesical wall exhibits rhythmic contractions which are reflected as regular variations of the intravesical pressure. From experiments on cats the phenomenon was described more than 70 years ago by Sherrington (1892), who also demonstrated such activity after spinal transection at different levels, after sacral rhizotomia and during the first 5—6 hrs following the death of the animal. Stewart (1900) extended Sherrington's observations in investigations of the bladder in vitro. He showed that spontaneous rhythmic contractions

were present in excised strips of the vesical wall placed in Ringer's solution Thus, the rhythmic activity of the animal bladder appeared to a large extent to be independent of the extrinsic nerves With respect to the human bladder, evidence of such independence was not reported until 1960 when Plum and Colfelt revealed the persistence of rhythmic vesical contractions, although somewhat reduced in amplitude, subsequent to spinal transections removal of the vesical plexus, and spinal anesthesia extending to thoracic levels These results, which were obtained with the aid of a highly sensitive pressure recording were carried further by Plum (1960), who found that rhythmic contractions were still present in the human bladder after injections of ganglionic blocking agents Hence, the statement was made that the urinary bladder, when distended contracts rhythmically due to the properties of the vesical wall It was categorically maintained that this activity was entirely unaffected by the vesical nerve supply, which is derived from the two divisions of the autonomic nervous system

Contrary to the opinion referred to above, most previous workers whether concerned with clinical studies or experimental work, advanced the opinion that the rhythmic bladder activity is dependent on nervous mechanisms With respect to the origin and mediation of these nervous effects different conclusions were reached (for systematic review of the literature, see Kuntz 1953 and Plum 1962)

In the clinic, the view has generally been held that injuries causing complete transection of the spinal cord or of the sacral nerves are followed by abolition of the rhythmic vesical activity By systematic studies of bladder motility subsequent to total transection of the spinal cord in patients with fractures of the spine Holmes (1933), Munro (1935), Prather (1953) Kollberg and Petersén (1963) noted a marked reduction of the spontaneous rhythmic contractions If the spinal cord lesion was situated cranial to the conus region the rhythmic muscular activity re appeared during the next weeks or months, and as a rule pressure fluctuations gradually increased in size far beyond the pressure peaks recorded from the intact human bladder Based on these observations the spontaneous rhythmic activity of the bladder was thought to be maintained by the effects of an autonomic sacral center The relaxation of the bladder initially seen was then explained as a result of spinal shock whereas the increase in rhythmic activity later occurring was ascribed to a loss of inhibitory influence from the cerebral level

Denny Brown and Robertson (1933) recorded powerful rhythmic contractions of the human bladder several weeks after injuries that interrupted all the sacral nerve roots In view of earlier observations Elliot 1907 that the rhythmic activity of the cat bladder did not re-occur subsequent to removal of the vesical plexus, the opinion was reached that nervous impulses which regulate the contractions of the bladder wall originate from autonomic ganglia within this plexus

Ample evidence was obtained by experimental work as well in support of an extrinsic nervous influence on the rhythmic bladder activity According to these observations such regulatory effects were conducted mainly through the parasympathetic nerves (Langworthy and Murphy 1939 Langworthy Kolb and Lewis 1940 Ingersoll and Hegre 1951 Carpenter and Root 1951

However Kuntz and Saccomanno 1944) also demonstrated an effect on the rhythmic bladder contractions by stimulation of the sympathetic vesical supply The response obtained was characterized by an initial sharp pressure peak followed by a fall in the pressure volume curve below the pre stimulatory level associated with abolition of the rhythmic activity

In spite of the dual autonomic nerve supply to the bladder, no data have so far been provided in favour of an antagonistic influence on the rhythmic vesical contractions. In the present investigation, performed in cats under light anesthesia, such evidence was searched for through partial bladder denervation. Thus parasympathetic effects on the rhythmic vesical activity were established from the changes which appeared in intravesical pressure records after bilateral section of this pathway alone. The role of the sympathetic bladder innervation was revealed by the pressure alterations recorded subsequent to primary bilateral section of this component. In both instances a comparison was made with the intravesical pressure reflected by continuous recording from the intact bladder and after deprival of the entire extrinsic nerve supply.

Material and methods

[illegible]

[illegible] previously described [illegible]

[illegible] controlled by a thermostat.

Identification and isolation of the different components within the vesical nerve supply. In the cat the [illegible]

[illegible] from the second and reach the vesical plexus through the pelvic nerves. The efferent [illegible] was interrupted by sectioning ventral spinal roots which were separated from the dorsal roots through an extensive laminectomy. To ensure complete denervation the seventh lumbar as well as the three sacral nerves were included in the procedure which was performed bilaterally.

Afferent fibres from the bladder accompany either of the two autonomic divisions. No attempt was made to isolate the afferent component of the sympathetic nerves because an additional thoraco-lumbar laminectomy is then required which would make it extremely difficult to maintain the animal in a good condition apart from the fact that complete section of the afferent pathway could hardly be secured despite extensive operative procedures. The afferent fibres of the parasympathetic supply were interrupted intraspinally by cutting the dorsal roots of the seventh lumbar and of the three sacral segments.

Results

In the intact and anesthetized animal rhythmic fluctuations of the intravesical pressure level were initiated by the first few ml of fluid introduced into the bladder cavity. During further distension of the vesical wall only a slight increase in frequency and amplitude of the rhythmic activity was noted up to the point of micturition. Commonly

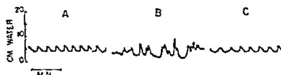


Fig 1 *A*, Rhythmic fluctuations of intravesical pressure at intact nerve supply, *B* Considerable changes in amplitude and frequency of the muscular activity of the same bladder at constant intravesical volume (20 ml) due to pain after fixation of the animal s head in a Horsley Clarke frame *C*, Normalization of the pressure record following relief of pain by local anesthesia (Cat 44)

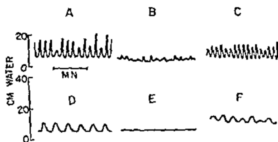


Fig 2 Intravesical pressure variations at constant bladder volumes Upper records Bladder filled with 20 ml saline solution *A* Intact nerves, *B*, Efferent parasympathetic nerve supply cut and *C*, in addition sympathetic supply bilaterally interrupted (Cat 73) Lower records Bladder filled with 10 ml fluid *D* Intact nerves *E*, Afferent parasympathetic nerves bilaterally sectioned and *F* Efferent parasympathetic nerves and sympathetic supply also cut (Cat 89)

a frequency of 3 to 5 contractions per min was recorded with an amplitude of the pressure variations seldom exceeding 10 cm water pressure

Individual differences were observed with respect to the frequency as well as the amplitude of the rhythmic pressure fluctuations, whereas a strikingly uniform activity was often seen in one and the same animal as long as the vesical nerve supply was not interfered with Such uniform contractions were in the present experiments recorded for periods of 3 to 6 hrs However, in some animals the rhythmic pressure fluctuations of the intact bladder were more or less irregular, particularly in amplitude

Considerable irregularities both in frequency and amplitude of the intravesical pressure undulations almost constantly ensued in animals showing signs of pain due to a too light anesthesia (Fig 1)

Since the recording method did not interfere with the bladder outlet, complete emptying of the vesical cavity was accomplished once the micturition reflex was released The protection thereby obtained against overstretching the contractile elements of the bladder appears from the fact that repeated filling procedures throughout hours in the acute experiments did not affect the typical features of the spontaneous vesical activity

Alternative bilateral interruption of the peripheral autonomic pathways to the bladder

1 The parasympathetic nerve supply Bilateral section of the spinal nerves L_7—S_3 was performed in 7 cats, leaving the sympathetic vesical supply completely intact The efferent (ventral) nerve roots only were cut in another group of 4 animals whereas primary bilateral section of the afferent nerve components at the same levels was carried out in 3 cats Essentially the same effects were obtained by these denervation

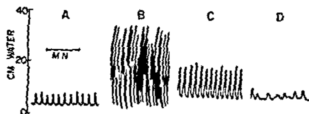


Fig 3 Rhythmic intravesical pressure variations at constant bladder filling (5 ml) A Intact nerve supply B immediately after section of the sympathetic chains C 15 min later and D, after interruption of the entire extrinsic vesical nerve supply (Cat 61)

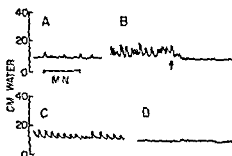


Fig 4 Changes in rhythmic vesical activity caused by pharmacological agents. A Bladder filled with 5 ml saline solution B intravesical fluid replaced by 5 ml 0 5 per cent

after replacing the fluid by the same volume of 0 05 per cent adrenalin (Cat 93)

procedures (Fig 2) The rhythmic activity of the intact bladder (Fig 2 A) is seen to be replaced by irregular contractions, profoundly reduced in amplitude (Fig 2 B

When a low degree of bladder distension is maintained the spontaneous contractions may even appear to be abolished (Fig 2 E) The final part of the pressure curves (C and E) which reflect bladder activity after an additional section of the sympathetic vesical nerves shows that the effect of parasympathetic denervation was counteracted although no complete restoration of the rhythmic contractions was obtained in the acute experiment

The marked changes in vesical activity following interference with the parasympathetic bladder innervation occurred immediately upon sectioning and remained the same during the entire period of observation which extended over 3—6 hrs

2 *The sympathetic nerves* Primary bilateral sympathetic denervation under maintenance of an intact parasympathetic supply was performed in 10 animals. Although not equally prominent in all experiments a marked increase of the rhythmic bladder activity usually ensued This applies to the amplitude as well as to the frequency of the contractions As a rule extreme effects occurred initially Fig 3 B Within the next 15—20 min there was a gradual decline particularly in frequency but also in amplitude Thereafter the rhythmic contractions remained fairly constant during the rest of the observation period 3 6 hrs still representing a considerable degree of hyperactivity Fig 3 C

In 5 cats complete interruption of the extrinsic autonomic vesical supply was performed through an additional section of the parasympathetic nerves The muscular activity of the vesical wall then immediately decreased and again regular contractions appeared closely resembling those recorded from the intact bladder although with some reduction in amplitude (Fig 3 D

Further evidence in support of a modulating antagonistic influence from the autonomic nervous system was provided through a preliminary study of changes in the rhythmic activity

(Fig 4 D)

Discussion

The present study of peripheral nervous influence on the rhythmic bladder activity was based on continuous pressure recording from the vesical cavity during step-wise bladder denervation After direct cannulation of the bladder through a cystostomy, leaving its urethral outlet intact (Gjone and Setekleiv 1963), the contractions of the muscular wall were reflected in the form of regular pressure fluctuations Following complete extrinsic denervation by cutting the parasympathetic as well as the sympathetic vesical supplies bilaterally, the rhythmic bladder activity remained essentially unaltered Thus evidence was obtained supporting the concept that the rhythmic vesical contractions are primarily autonomous in character, as also emphasized by a minor group of recent investigators (see Plum 1962) Doubts are, however, raised when these workers, from the same data argue that the rhythmic pressure variations are merely reflecting an inherent property of vesical smooth muscle, which is not in any respect affected by extrinsic peripheral nervous mechanisms. This statement, it is believed was made because they were preoccupied with the similar patterns of vesical activity recorded from the intact bladder and after complete extrinsic denervation Apparently they have thereby overlooked the conspicuous effects induced by partial autonomic denervation revealed in the present experiments. These observations demonstrate the existence of a peripheral nervous mechanism which exerts a modulating tonic influence on the smooth muscle activity within the vesical wall This conclusion is in accord with the view generally held that the rhythmic activity of the vesical wall is subordinated nervous regulation The present study does not however yield support to the common concepts about the role of the different autonomic systems in mediating such nervous effects to the bladder As mentioned in the introductory part both excitatory and inhibitory influence on vesical smooth muscle were by previous investigators attributed to the parasympathetic bladder innervation whereas no important effects were found to be conducted by the sympathetic pathway Thus according to Learmonth (1931) the activity of the detrusor muscle remained unaltered after section of the hypogastric nerves as judged from cystoscopic examination Similarly Langworthy *et al* (1940) observed no effect on the intravesical pressure fluctuations by bilateral section of the sympathetic chains In the present experiments sympathetic bladder denervation was found to cause a marked increase both in frequency and amplitude of the rhythmic bladder contractions, which on the other hand were abolished or greatly reduced by isolated parasympathetic denervation Thus an antagonistic autonomic mechanism is shown to regulate the rhythmic bladder activity by excitatory parasympathetic and inhibitory sympathetic effects on vesical smooth muscle The discrepancy between earlier observations and the results obtained in this work might possibly be explained by methodological considerations Thus, it seems likely that the present observations were facilitated to some extent at least by a better protection of the contractile elements within the vesical wall due to the maintenance of an open outlet from the bladder cavity

Further evidence of a dual autonomic regulation of the rhythmic bladder contractions was provided by the results following stimulation of the peripheral vesical nerves

Acta physiol. scand. 1965, 65, 378—382

From the Central Hospital of Savonlinna, Savonlinna and from the Department of Pathology Section II, University of Helsinki Finland

Hydrogen Ion Concentration in the Gastric Juice after Pylorus Ligation in Dexamethasone-Treated Rat

By

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Received 6 May 1965

Abstract

HAIKONEN, M. and T. RÄSÄNEN. *Hydrogen ion concentration in the gastric juice after pylorus ligation in dexamethasone treated rat.* Acta physiol. scand. 1965. 65. 378—382. — Rats were injected with dexamethasone 5—1 mg at 12 hr intervals before ligation of the pylorus. The hydrogen ion concentration of the gastric secreta, the volume of secretion and the amount of free HCl were measured 2 hrs after tying the ligature. The administration of dexamethasone for 2 days before ligation of the pylorus inhibited gastric secretion during the ligation and elevated the pH of the secreta. No free HCl was demonstrated in it. Secretion was restored by histamine injected into the rats in connection with the application of the ligature. A single dose of dexamethasone 2 hrs before the application of the ligature caused elevation of gastric secretion.

It is suggested that the degranulation of the mast cells of the gastric mucosa during dexamethasone therapy leads to inhibition of gastric secretion, with the mucosa losing the histamine needed for its stimulation. This loss may be compensated by exogenous histamine. On the other hand, the histamine liberating property of dexamethasone enhances secretion when the degranulation of the mast cells begins.

The histamine which appears in the gastric juice when the stomach is stimulated by various means is a peripheral stimulant of the parenchyma of the stomach (Code 1956). The histamine content rises in portal blood (Silen and Eiseman 1961) and in urine (Irvine and Code 1958) during gastric secretion. The gastric mucosa contains much histamine (Douglas *et al.* 1951) which in rat gastric mucosa decreases together with the degranulation of mucosal mast cells during cortisone therapy (Foley and Glick 1962).

A rapid vagal and slow endocrine component were distinguished by French *et al.* (1953) among the agents stimulating gastric secretion in rhesus monkeys. Both components are probably activated in the gastric stimulation provoked by pylorus ligature and this is followed in 16—18 hrs by lesions to the rumen of the rat (Shay *et al.* 1945). The damage is probably caused partly by a vascular disturbance due to elevated intraluminal pressure (McArthur and Michel 1960). Vagotomy does not prevent lesions in the gastric mucosa of Shay ligated rats (Sundell and Leir 1956).

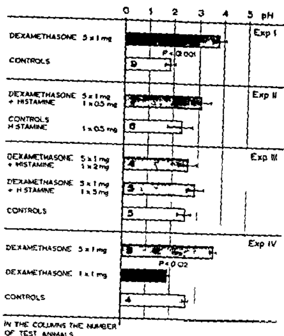


Fig 1 The columns indicate means ± standard errors of hydrogen ion concentration in the gastric juice

Dexamethasone degranulates mucosal mast cells very effectively (Rasanen 196?) It is possible that the histamine they contain transmits the peripheral stimulation of the gastric parenchyma and their degranulation may be associated with gastric secretion (Rasanen 1964) Inhibition of secretion by Shay ligature is possible after dexamethasone management Elucidation of this question is the object of the present study

Method

Male rats of Dawley-Sprague strain were used in the study The rats which were 4—5 months of age received for 2 —4 weeks rat food (Hankkija) corresponding to Purina A and water The dexamethasone treatment lasted for 48 hrs during which the rats were injected with 5 1 mg of dexamethasone (Dexa Scheroson Schering Leiras) at 12 hr intervals The last injection was 2—4 hrs before ligation of the pylorus The pylorus ligature was applied under light nembutal ether anesthesia during laparotomy 2 hrs after tying the ligature the rats were killed by exsanguination under ether anesthesia the stomach was dissected free and its contents emptied into a small dish The pH of the gastric secreta was determined immediately potentiometrically The secreta were then separated in a part of the studies from the solid gastric contents by vacuum filtering the volume was measured and the amount of free HCl was determined by titration with Topfer's reagent as the indicator During the dexamethasone injection period a part of the rats were kept on an Aminosol-glucose diet to avoid solid gastric contents The composition of the Aminosol-glucose solution was
30 parts of Aminosol 10 per cent Vitrum"
20 parts of glucose solution 50 per cent
3 parts of Liq Darrow II

The physiologic combustion value of the nutrient solution was 1 kcal/ml. The test animals were given the solution ad libitum.

Fisher's "Student's" t test was used in the statistical treatment of the results.

Results

The study was divided into 4 different experiments. The results of each are presented separately. In the first experiment (Fig. 1), 15 rats received dexamethasone prior to the pylorus ligation and 9 rats from the control group received no injections before the application of the ligature. Ten of the former and 5 of the latter were given Aminosol glucose during the test period, the others received rat feed. A distinct difference was seen in the pH of the various groups; it was higher for the rats receiving dexamethasone than for the controls. It was 3.9 ± 0.5 with ordinary rat food and dexamethasone, 1.8 ± 0.2 for the controls. The comparable values for the rats on Aminosol-glucose were 3.73 ± 0.7 and 1.9 ± 0.3.

In the second experiment, 0.5 mg of histamine dihydrochloride was injected into the closed peritoneal cavity after ligating the pylorus. The rats given dexamethasone had a higher pH than the controls, but the difference was not significant statistically.

In the third experiment, 1.0 and 5.0 mg of histamine was injected into the peritoneal cavity of the rats given dexamethasone. No manifest deviations were stated in the hydrogen ion concentration in this experiment. The secretion volumes and the free HCl content were as follows (range in brackets):

	Volume of secretion	Free HCl 0.1 n 100 ml
Dexamethasone + histamine 1.0 mg	1.6 ml (1.2 – 2.1)	71 (3 – 111)
Dexamethasone + histamine 5.0 mg	2.1 ml (2.0 – 3.5)	117 (4 – 225)
Controls	3.6 ml (0.8 – 5.2)	7 (2 – 92)

A large quantity of histamine appeared to cause profuse secretion of HCl in the gastric mucosa of rats given dexamethasone.

In the fourth experiment, 48 hr dexamethasone therapy lowered the gastric secretion but if the dexamethasone was administered only once the pH of the gastric juice fell distinctly in connection with pylorus ligature. The volume of the secretion and the free HCl content of these rats were as follows (range in brackets):

	Volume of secretion	Free HCl 0.1 n 100 ml
Dexamethasone 5 × 1.0 mg	2.8 ml (1.5 – 5.5)	Not obtained
Dexamethasone 1 × 1.0 mg	4.0 ml (2.5 – 6.0)	57 (46 – 71)
Controls	4.2 ml (2.0 – 2.8)	42 (12 – 50)

Dexamethasone administered in a single injection seems to have a stimulating effect on gastric secretion.

The gastric contents of the rats contained small amounts of blood in some cases obviously due to a lesion caused by the ligature. Mucosal hyperemia was established in all the rats; manifest ulceration did not appear to arise in the mucosa within 24 hours.

of the application of the ligature The stomach was full of secretion in some cases, but not distended enough for the pressure of its contents to cause circulatory disturbances in the wall of the stomach

Discussion

The volume of free HCl decreased and the pH rose distinctly in rats given dexamethasone for 48 hrs in connection with Shay ligature Dexamethasone degranulated the mast cells of the mucosa of the fundus and the pylorus very effectively (Rasanen 1962, 1963) The histamine content of gastric mucosa decreases in connection with degranulation (Foley and Glick 1962) On the other hand, dexamethasone application commenced at the [illegible]

of lesions in the rat gastric mucosa especially after adrenalectomy (Peremans 1958) when the quantity of the histamine (Bartlett and Lockett 1959) and of the mucosal mast cells in the gastric mucosa is elevated (Rasanen 1961) This peripheral histamine contained in mast cells probably stimulates the parenchyma of the stomach after adrenalectomy with cortisone restoring gastric secretion to normal or even above normal depending on the dose (Nicoloff *et al* 1961)

Dexamethasone causes loss of intracellular potassium (Pechet 1962) Potassium appears especially in the initial phase of secretion in the gastric juice, but its content is not needed for the secretion during histamine stimulation (Werther and Hollander 1962) On the [illegible] side of the m[illegible]cosa the increase in the potas[illegible]

[illegible] jected histamine seems to be able to make good the endogenous histamine deficit and to return the gastric secretion to the same level as in the controls

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Influence of the Nutritional State on the Inhibition of Lipolysis in Adipose Tissue by Prostaglandin E_1 and Nicotinic Acid

Prostaglandin and related factors 46

By

SUNE BERGSTRÖM and LARS A CARLSON

Prostaglandin E_1 (PGE_1) inhibits lipolysis in rat (Steinberg *et al* 1964) and human (Bergström and Carlson 1965, Carlson 1965) adipose tissue incubated *in vitro* Nicotinic acid also inhibits this lipolysis in rat (Carlson 1963 Eaton 1963) and human (Carlson 1965) adipose tissue *in vitro* The nutritional state has a significant regulatory effect on the mobilization of lipids from adipose tissue in the intact organism, the rate of mobilization increasing with fasting The exact nature of the process(es) and factor(s) stimulating lipid mobilization in fasting is not known The present study was undertaken to see if PGE_1 and nicotinic acid had any effect on the lipolysis in the fasting state

Sprague-Dawley rats (AB Anticimex, Stockholm) weighing 180 g were used The rats were decapitated at around 10—11 a m , the epididymal fat pads cut into pieces and randomized between the incubation flasks according to the pooled adipose tissue technique of Froesch *et al* (1963) as modified and described in detail earlier (Carlson 1965 Bizzi and Carlson 1965) Krebs Ringer bicarbonate buffer with 2 per cent human albumin (AB Kabi Stockholm) and 0 1 per cent glucose was used as incubation medium

Table I shows that PGE_1 as expected inhibited the release of glycerol from adipose tissue of fed rats at a concentration of 0 01 μg/ml incubation medium However when PGE_1 was added *in vitro* to adipose tissue of starved rats in concentrations varying from 0 001 to 1 μg/ml incubation medium no significant inhibition of the glycerol release was obtained In two of these studies (Exp 2 and Exp 8) even a stimulation of the glycerol release which was of borderline statistical significance, was observed

Nicotinic acid on the other hand inhibited the glycerol release from both fed and fasted rat adipose tissue Also with this agent, however, did the nutritional state influence the inhibition The inhibition of lipolysis was much more pronounced in the fed than in the fasted state Table I

The resistence of lipolysis in the fasting state towards the inhibitory effect of PGE_1 suggests that PGE_1 exerts no influence in the regulation of lipolysis in adipose tissue in the transition from the fasting to the fed state Any interpretation of these findings has to await a better basic knowledge of the effects and interplay of various hormonal and nutritional factors on the lipolysis during fasting

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TABLE I Effect of PGE_1 (Exp 1—8) and nicotinic acid (Exp 9—10) on the release of glycerol (μmoles/g adipose tissue/hr) from adipose tissue of rats fed *ad libitum* and of rats fasted for 18 hrs Mean value ± standard error of the mean and number of incubation flasks

		Concentration of added agent μg/ml medium	0	0.001	0.01	0.1	1
PGE_1	Fed rats	Exp 1	2.65±0.12 7	2.66±0.15 6	1.85±0.20 6	1.57±0.27 6	—
		Exp 3	1.98±0.09 5	—	—	1.03±0.04 6	1.14±0.07 5
		Exp 5	2.10±0.04 7	1.60±0.06 7	1.29±0.07 6	0.89±0.03 6	0.81±0.04 6
		Exp 7	1.95±0.17 8	—	—	—	1.14±0.06 8
	Fasted rats	Exp 2	2.35±0.13 7	2.81±0.07 6	2.52±0.15 5	2.53±0.17 6	2.15±0.21 7
		Exp 4	2.12±0.09 5	—	—	2.15±0.06 5	1.91±0.07 6
		Exp 6	1.69±0.13 7	1.82±0.03 7	1.62±0.13 6	1.54±0.14 6	1.51±0.07 6
		Exp 8	1.97±0.10 8	—	—	—	2.29±0.10 8
Nicotinic acid	Fed rats	Exp 9	2.09±0.06 5	-	—	0.60±0.06 5	0.58±0.04 5
	Fasted rats	Exp 10	1.99±0.07 5			1.22±0.05 6	1.35±0.07 5

On a given day rats from the same batch one fed and one fasted group were studied These two groups have been given consecutive experimental numbers in the table

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